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The Effects of Exercise on Pharmacokinetics and Pharmacodynamics of Physostigmine in Rats

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This report describes the effects of a single dose of physostigmine (Phy) on the ability of rats to exercise, effects of prior exercise on acetylcholinesterase (AChE) kinetics, and the effects of exercise training on AChE kinetics.

Dose-response studies of Phy and cholinesterase (ChE) inhibition in vivo was carried out. Based on dose-response curves, we selected a dose of 70 ug/kg of Phy, which will inhibit about 30% ChE in red blood cells (RBC). This dose was used for our exercise studies.

A comparison of oxygen consumption and heat production in young and adult rats indicated that young rats attained a higher VO_2 max (81.55 ml/kg/min) than the adult rats did (68.97 ml/kg/min). The younger rats showed a higher resting heat capacity (11.84 kcal/kg/hr) than the adult rats did (8.53 kcal/kg/hr). Different intensities of acute exercise (50%, 80% and 100% VO_2 max) produced a significant inhibition of ChE activity in heart and in

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diaphragm without significantly affecting brain and thigh muscle. However, acute exercise produced a slight increase in RBC ChE activity. Phy inhibited ChE in RBC and in all the tissues studied. Phy followed by acute exercise further increased the ChE inhibition in TBC and brain without affecting heart, diaphragm or thigh muscle. Phy administration followed by acute exercise resulted in a significant decrease in lactate and pyruvate concentration. Phy combined with acute exercise decreased the increase in hemoglobin induced by exercise alone. Phy (70 ug/kg, i.m.) increased the endurance time of rats weighing 160-200 g.

Exercise training of rats for 6 weeks slightly decreased the ChE activity. Training and Phy administration further decreased the ChE activity in RBC, brain, heart, diaphragm and thigh muscle, indicating that training increased the Phy-induced ChE inhibition. Exercise training and Phy interaction seem to affect the lactate and pyruvate metabolism within 30 min after exercise.

SUMMARY

This report provides information on the dose response of physostigmine (Phy) in in vivo system. It was essential to conduct dose-response studies of Phy in order to determine the dose which would produce 30% ChE inhibition in RBC; we needed this information for the exercise experiments.

Dose response of Phy was studied in rat using various doses (25-500 g/kg, i.m.). Rats were sacrificed 15 min after Phy administration. RBC and tissues were analyzed for ChE activity by the radiometric method and for Phy concentration by the high-performance liquid chromatograph (HPLC) method. A comparison of ChE values in different tissues of rats indicated that ChE activity was highest in brain (7.11 mol/min/g) and lowest in diaphragm (0.67 mol/min/g). The enzyme activity was 11 times higher in brain than in diaphragm. From 50 to 200 g/kg, Phy produced a dose-dependent inhibition of ChE in RBC (18-42%), brain (23-55%) and diaphragm (25-35%); then ChE inhibition plateaued at 200-500 g/kg in these tissues. A dose related ChE inhibition was seen in heart (16-50%) and thigh muscle (8-53%) at 50-500 g/kg. Phy concentration increased linearly at 50-400 g/kg in plasma, brain, heart and thigh muscle. These results indicate that ChE inhibition is linear up to 200 g/kg in RBC, up to 150 g/kg in brain and up to 300 g/kg in heart. This linearity is not consistent in other tissues.

As a first step in our exercise study we have compared oxygen consumption, respiratory exchanges ratio (RER) and heat production in young and adult rats at different exercise levels. Open-circuit indirect calorimetry was used to determine metabolic variables in young (147 $\pm$ 2 g) and adult (332 $\pm$ 7 g) rats undergoing identical acute exercise regimens on a motor-driven treadmill with a computerized Oxyscan System. The animals were run within enclosed chambers with a positive air flow rate of 3650 ml/min using an incremental exercise protocol in order to compare oxygen consumption (VO_2), RER and heat production. The resting VO_2 was significantly higher in young (40.80 ml/kg/min) than in adult (29.23 ml/kg/min) rats. The young rats attained a higher VO_2 (81.56 ml/kg/min) than did adult rats (69.98 ml/kg/min) at a maximal level of exercise (VO_2 max). Based on unit mass, the young rats had a higher heat capacity, both at resting (11.84 kcal/kg/hr) and at VO_2 max (24.11 kcal/kg/hr) than did adult rats. There was a positive linear relationship between VO_2, RER and heat production at different levels of acute exercise in both groups of rats. These data show that similar responses relative to VO_2, RER and heat production occur during acute exericse in young and adult rats with the only difference between the two groups being a greater metabolic response in the young than in the adult rats.

We have studied the effects of Phy and concurrent acute exercise on the ChE activity in RBC and tissues and in blood biochemical parameters in rats. Phy has been reported to reduce the endurance the endurance time and increase the rate of rise of core temperature in rats. This report explains the effects of the following on ChE activity in RBC and tissues of male Sprague-Dawley rats (160-200 g): i) three different levels of acute exercise (50%, 80% and 100% VO_2 max); ii) administration of Phy (70 g/kg, i.m.); and iii) Phy administration immediately followed by three different levels of acute exercise. ChE was determined by the radiometric method. Different intensities of acute exercise (50%, 80% or 100% VO_2 max) did not show significant differences in ChE activity in RBC, brain and thigh muscle. Acute exercise and Phy produced more ChE suppression in RBC and brain than did Phy alone. In contrast, Phy alone caused more ChE suppression in thigh muscle, heart and diaphragm than did Phy and acute exercise.

Physostigmine administration followed by acute exercise at 80% and 100% VO_2 max significantly decreased the plasma lactate concentration (144% and 179% of control) as compared to exercise alone (180% and 219% of control). These results indicate the interaciton of Phy and acute exercise on lactate metabolism. Acute exercise at 80% and 100% VO_2 max increased plasma pyruvate concentration. At 100% VO_2 max, Phy and acute exercise decreased the plasma pyruvate content as compared to acute exercise alone. Different intensities of acute exercise slightly elevated the hemoglobin content. Phy administration followed by acute exercise increased the hemoglobin content compared to exercise alone. Significant change in hematocrit value was not observed. Phy (70 g/kg, i.m.) and acute exercise increased endurance time.

For exercise training of rats, we fabricated a 9-channel treadmill at our research workshop at Southern Illinois University (SIU) School of Medicine, Springfield, Illinois. Also, we have studied the effect of exercise training, Phy and training + Phy on ChE activity in RBC and tissues and on blood biochemical parameters in rats. Male Sprague-Dawley rats (160-200 g) were exercised for 6 weeks (5 days per week) in a 9-channel motor-driven treadmill. Rats were sacrificed at 2, 5, 10, 15, 30, 45 and 60 min after exercise training (gr. 1), after Phy administration (70 g/kg, i.m.) (gr. 2) and after exercise training immediately followed by Phy adminstration (gr. 3).

Exercise training of rats slightly decreased ChE activity in RBC, brain, heart, diaphragm and thigh muscle from 5-60 min. Phy administration (70 g/kg, i.m.) depressed the ChE activity in RBC and tissues up to about 10 to 30 min, which was recovered within 60 min. The training and Phy administration further depressed the ChE activity up to 30 min, which slowly recovered within 60 min, indicating that the training increased the Phy-induced ChE inhibition.

Exercise trainging and Phy interaction seem to affect the lactate and pyruvate metabolism within 30 min after exercise. Normal steady states were reached after 30 min, indicating the time taken for returning to normal state after exercise training and drug administration. The results of our exercise studies indicate that exercise training will help the body to cope with the stress conditions of Phy administration and will help to restore it to normal condition sooner than when no exercise is performed.

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

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INTRODUCTION

Physostigmine (Phy), an anticholinesterase drug, is considered to be a useful pretreatment drug for protection against organofluorophosphate agents (1-8). The rates of its absorption, distribution, metabolism and excretion are most important in determining the protective action of Phy, as is its ability to inhibit ChE activity (9-11). The pharmacodynamics of Phy (cholinesterase activity) are likely to be altered by exercise due to altered blood flow rates to liver and pH of muscle. During exercise, cardiac output increases with the intensity of workload, and concomitant changes in regional blood flow distribution occurs. Thus, blood flow to skeletal muscles and skin is greatly increased, while, on the other hand, hepatic blood flow decreases during exercise (12,13).

Recently, Francesconi et al. (14) reported the effects of Phy (ChE inhibition of 64%) on the ability of rats to work in the heat. Phy-treated (200 /kg) rats weighing about 500 gm had a mean endurance time of 23 min, whereas saline-treated animals ran for nearly 35 min.

Exercise basis:

The exertional demands of modern military duty may range from levels that are not much different from those required for most civilian tasks to the more strenuous requirements of the infantry soldier (15). Combat-oriented tasks have been char-acterized as involving basic body efforts such as lifting, carrying, climbing, pushing, pulling and torquing (16), which may require a range in metabolic equivalents (17) from several METS to 12 METS for very heavy short-term work (1 MET is equivalent to 1 kcal/kg x hr). In expenditure situations which would require rapid prolonged marching, an energy expenditure of 3-5 METS for sustained periods (18) would be required, while heavy rescue work could be expected to require a heavier energy expenditure demanding an oxygen consumption of about 3.0 l/min (8-12 METS) (19). At the present time there is little ergonomic data to specifically classify many of the specific job tasks of the combat soldier. However, it is reasonable to assume that a range of exertion from rest to 80% of work capacity, as measured by maximal oxygen consumption (20), would adequately encompass the majority of the physical requirements of these personnel. The muscularly demanding tasks of the combat infantry soldier can be expected to result in significant physical and chemical changes within the body (21-23). It is also known that physical training improves the efficiency of these processes when the individual subsequently engages in physical exertion (24-27). This is the end to which much of the physical preparation for military personnel is directed during basic and supplemental training (28-30). Notwithstanding this, physiological stress is still to be expected, causing a redistribution of blood flow to serve the demands of the active muscle cells (31, 32) as well as to meet the needs of temperature regulation. This is especially true during prolonged submaximal (33-35) exercise. In addition, a considerable production of metabolic acidosis from substrate catabolism will lead to a marked reduction of the intracellular pH (36-38). Since the time course of a drug may be influenced by exercise dynamics (39, 40), it is important to know how exercise interacts with a drug which would potentially be administered under combat field conditions. This information would be of special consequence for a drug such as Phy, a tertiary amine, administered as a pretreatment drug in chemical defense (41). It is the intent of this research to simulate military conditions using an animal model. Therefore, chronic training similar to that described by Armstrong et al. (42) as well as short-term steady-rate exercise (43) was used. Short-term work will approximate 60% and 80% of peak oxygen consumption to represent moderate and

strenuous work (44). In this way, a simulation of the military tasks involved in daily normal activities, rapid marching and combat duty will be reasonably covered.

The rat as an animal model has been previously used to study the activity of neurotransmitters during exercise (45, 46) as well as under other stressful conditions (47-49). The effects of drugs combined with other stressors on exercise performance have also been studied using the rat as the experimental animal (50, 51). In a more recent study, Francesconi et al. (14) studied the effects of physostigmine on the ability of rats to work in the heat. In this study, the drug was found to reduce endurance time, to increase rectal and skin temperatures and, possibly, to contribute to the pathophysiology of exercising muscle as evidenced by an increase in serum creatine phosphokinase measured after the exercise. Since these animals were exercised at an ambient temperature of 35°C, it is not known whether similar endurance and biochemical responses would occur in physostigmine-treated rats subjected to prolonged exercise in a thermally neutral environment. In addition, exercise is associated with the production of lactic acid (52), metabolic acidosis (53), and changes in hemoglobin, hematocrit, and plasma volume (54-56).

This report describes the effects of acute exercise and training and physostigmine on i) ChE activity in RBC, brain, heart, diaphragm and thigh muscle in rats ii) biochemical parameters such as pyruvate, lactate, hemoglobin and hematocrit in rats. This report will also provide information on the comparison of the kinetics of dose response of physostigmine in in vivo and in in vitro systems. The dose-response curve of physostigmine has provided us the information on the appropriate dose to be used in exercise experiments, i.e., the dose which will produce 30% inhibition of ChE in RBC. In addition to this general introduction, a brief introduction is given to each section.

I. IN VIVO DOSE-RESPONSE RELATIONSHIP BETWEEN PHYSOSTIGMINE AND CHOLINESTERASE ACTIVITY IN RBC AND TISSUES OF RATS.

Introduction:

The disposition of physostigmine (Phy) in rat after i.m., i.v. and oral administration was studied by Somani and Khalique (9,10) and Somani (11). These investigators have also determined the time course of cholinesterase (ChE) inhibition in plasma, brain and muscle and have correlated i.e., with Phy concentration after i.m., i.v. and oral routes of administration. ChE inhibition in six different rat brain regions was also shown after i.m. and i.v. administration of Phy (57,58). ChE activity is an important parameter in monitoring the efficacy of Phy. ChE inhibition in blood has been studied in mice (59), rabbit (5,60), rat (8,60,61) and guinea pig (60). These authors have estimated only the whole blood ChE enzyme for determining the dose response of Phy. There is a paucity of data on ChE inhibition in different tissues and on the relationship between ChE inhibition and Phy concentration. The present study examines the effects of different doses of Phy on ChE activity in RBC, brain, heart, diaphragm and thigh muscle and correlates Phy concentration in these tissues to the ChE activity.

Materials and Methods:

Physostigmine free base was obtained from Sigma Chemical Co. (St. Louis, MO). ^{3}H-Phy (13 Ci/mmol) was custom-synthesized by Amersham Corp. (Chicago, IL). Methanol (HPLC grade) was obtained from Burdick and Jackson Laboratories, Inc. (Muskegan, MI). Ready-Solv EP was obtained from Beckman Instruments, Inc. (Fullerton, CA) and Monophase-40 plus was from Packard Instrument Co. (Downers Grove, IL). All other chemicals were analytical grade and were obtained from the usual commercial sources.

Preparation of ^{3}H-Phy solution:

Phy solution was prepared in 0,9% (w/v) saline to which 10 ul of hydrochloric acid was added to keep the Phy solution acidic (pH 3.5). Phy appears to be more stable in acidic solution. ^{3}H-Phy (200 uCi/100 ul of 13 Ci/mmol) was added to unlabelled Phy (free base) to obtain the final concentrations of 50, 200, 300 and 400 ug/ml. Radioactivity in each dose was determined by liquid scintillation counter and was found to be 84.4 uCi/50 ug/ml, 149 uCi/200 ug/ml, 123.5 uCi/300 ug/ml, and 145.5 uCi/400 ug/ml.

Dosing and sacrifice:

Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 175-250 g were used. A group of 6-8 rats was administered various doses of Phy (25 ug/kg-500 ug/kg) intramuscularly in the right thigh (dorsal surface). Control rats (10-12) were given saline. The animals were sacrificed by decapitation at 15 min after dosing. Blood was collected in heparinized tubes. Brain, diaphragm, heart and thigh muscle (left side) were removed, rinsed with ice cold saline and blotted dry. All samples were stored at -70°C until analysis.

Preparation of blood samples:

Blood plasma was separated by centrifugation at 2000 rpm for 10 min at 40°C in Sorvall RC-5 centrifuge (DuPont Instruments Co., Irving, TX) and stored at rocytes (RBC) were separated by pouring blood (0.5 ml) slowly, drop by drop,

into a 15 ml glass tube containing 10 ml ice cold saline, centrifuging at 2000 rpm for 10 min at 4°C and aspirating off as much of the supernatant as possible. The suspension of erythrocytes was prepared by adding 2 ml of 0.1 M phosphate buffer (containing 1% Triton X-100 and 0.9% NaCl) and shaking the contents on a vortex mixer for 15 sec.

Preparation of tissue homogenates:

Tissues were weighed, minced and homogenized in buffer (0.1 M phosphate buffer, pH 7.2, 1% Triton X-100, 0.9% NaCl) using a Polytron homogenizer (Kriens, Switzerland); the tubes were cooled as needed. A homogenate of 10% for brain, diaphragm and thigh muscle and 5% for heart was prepared. The homogenates were centrifuged at 10,000 rpm for 10 min at 4°C. The clear supernatant was used for ChE determination.

Determination of the ChE activity:

The ChE enzyme estimation was carried according to a modification of the radiometric method of Johnson and Russell (62). In this procedure, ^{3}H-ACh is used as the substrate. The radioactivity (RA) due to ^{3}H-acetate formed by the enzymatic hydrolysis of ^{3}H-ACh was measured by this method. The substrate was prepared fresh daily by mixing 0.5 M Tris buffer (0.25 M Trizma base, 0.25 M Tris-HCl, 1.2 M NaCl, pH 7.4), AChCl (0.1 mmol for RBC, diaphragm, heart and thigh; 1 mmol for brain) and ^{3}H-AChI (1 mCi/0.0100 mmol).

Fifty-microliter aliquots of RBC suspension and 50 ul of phosphate buffer were put in glass scintillation vials and then 50 ul of freshly prepared ^{3}H-ACh solution (2 umol/1 uCi) was quickly added to each vial. The total volume of reaction mixture was 150 ul. For diaphragm, heart and thigh muscle, aliquots of 50 ul of homogenate and 30 ul phosphate buffer were taken and then added to 20 ul of ^{3}H-ACh solution (2 umol/1 uCi), the total volume of reaction mixture being 100 ul. Brain ChE activity was determined by taking 50 ul of brain homogenate, 50 l of ^{3}H-ACh solution (1 umol/1 uCi) and 50 ul of phosphate buffer: the total volume of reaction mixture was 150 ul. All the estimations were carried out in triplicate. Blanks representing the non-enzymatic hydrolysis of ACh were also prepared in triplicate with each sample run and subtracted off as background. The reaction mixture was incubated at 37°C for 15 min in Thermolyn waterbath and the contents were immediately cooled on ice and stop solution (100 l) was added to check the enzymatic hydrolysis. Then 4 ml of toluene scintillation cocktail (0.51% PPO, 0.03% POPOP and 10% isoamyl alcohol) was added to each sample. Ready-Solv (Beckman, Fullerton, CA) was used for measuring specific activity of the substrate. The samples were mixed on a vortex and the RA was counted in a Beckman LS 5800 Liquid Scintillation Spectrometer.

The ChE values of RBC are expressed as umol of ACh hydrolzed/min/g of hemoglobin content, whereas the tissue ChE values are expressed as umol of ACh hydrolyzed/min/g of wet weight of tissue.

Determination of hemoglobin:

The hemoglobin content of blood was determined by Sigma diagnostic kit using a Beckman Spectrophotometer at 540 nm.

Determination of Phy in plasma and tissues:

Physostigmine in plasma, brain, heart and thigh muscle was determined by HPLC following the method of Somani and Khalique (9,63).

Statistical analysis:

ChE activity and Phy concentration were analyzed using Student's "t" test to identify the significant differences in the individual experiments. A significance level of $P < 0.05$ was used.

Results:

A comparison of the cholinesterase values in different tissues in rats indicates that the ChE enzyme activity was highest in brain and least in diaphragm (Table 1). The enzyme activity was 11 times higher in brain than in diaphragm. The relative magnitudes of ChE activities in tissues were as follows: brain > heart > thigh > diaphragm. Phy concentration in plasma, brain, muscle and heart was determined by HPLC and the radiometric method. For all dosages, brain concentrations were highest followed by plasma, muscle and heart.

Effect of various dosages of Phy on ChE activity in RBC and tissues and on Phy concentrations:

RBC:

Physostigmine produced a dose-dependent reduction of RBC-ChE activity when given in dosages from 25 to 200 ug/kg (Fig. 1). The ChE inhibition values were 3%, 18%, 31%, 37% and 42% with 25, 50, 100, 150 and 200 ug/kg ofPhy, respectively. However, the inhibition with 25 ug/kg was statistically insignificant as compared to control values. The percentage inhibition plateaued at higher dosages of Phy (300, 400 and 500 ug/kg). The ChE inhibition was found to be 39%, 35% and 36% with 300, 400 and 500 ug/kg of Phy, respectively. At these higher doses (300-500 ug/kg), ChE inhibition did not increase with increasing dose and the average level of inhibition was less than the maximal inhibition (I_{max}) observed at a relatively lower dose (200 ug kg). Concentration of Phy in the plasma, on the other hand, showed a linear increase with increase in dose; the plasma concentrations of Phy at 50, 200,300 and 400 ug/kg dose were 12.69, 47.02, 70.74 and 105.84 ng/ml, respectively.

Diaphragm:

Phy produced a dose-dependent inhibition of diaphragm ChE from 25 to 100 ug/kg, the inhibition values being 18%, 25% and 42% with 25, 50 and 100 ug/kg of Phy (Fig. 1). However, an increase in dose from 150 to 400 ug/kg of Phy produced a slight decrease in % ChE inhibition as compared with inhibition at 100 ug/kg. This decrease appeared to be statistically insignificant. Phy in a dose of 500 ug/kg produced increased ChE inhibition (44%), which was similar to inhibition at a 100 ug/kg dose. It is difficult to explain these swings in ChE inhibition with different dosages.

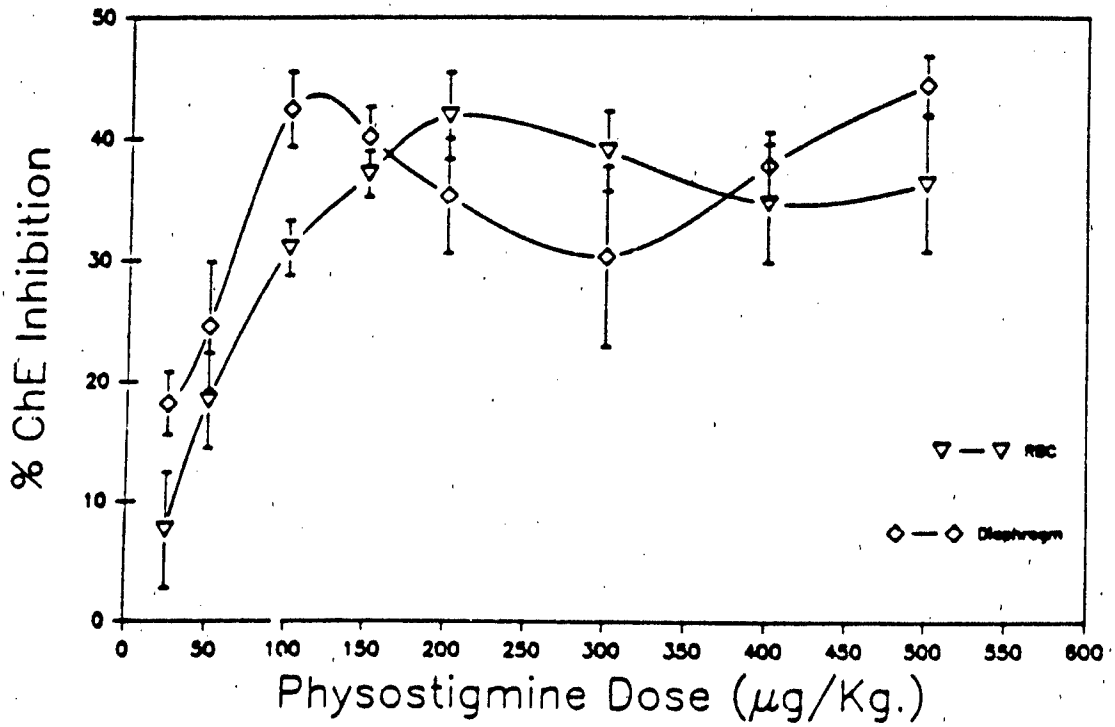

Figure 1: Effect of i.m. administration of physostigmine (25-500 ug/kg) on % ChE inhibition in RBC and diaphragm of rats. Each point is the mean ± S.E.M. of 6-8 rats.

Table 1: Effect of various doses of physostigmine on cholinesterase activity in RBC and tissues of rats

IM Phy Dose (ug/kg)	RBC ChE umole/min/g of Hb	Tissue ChE umole/min/g			
		BRAIN	HEART	DIAPHRAGM	THIGH MUSCLE
-	2.103±0.141	7.110±0.256	1.706±0.134	0.672±0.045	0.820±0.036
25	1.944±0.121	7.248±0.212	1.986±0.070	0.550±0.018	0.790±0.037
50	1.716±0.084[a]	5.489±0.369[b]	1.434±0.136	0.507±0.036[b]	0.751±0.029
100	1.451±0.048[c]	2.758±0.276[c]	1.238±0.109[a]	0.387±0.021[c]	0.495±0.042[c]
150	1.324±0.039[c]	2.401±0.248[c]	1.182±0.031[b]	0.402±0.017[c]	0.478±0.023[c]
200	1.223±0.075[c]	3.209±0.114[c,e]	1.045±0.037[c]	0.435±0.032[c]	0.475±0.033[c]
300	1.248±0.069[c]	3.619±0.120[c,d]	0.908±0.040[c]	0.468±0.050[b]	0.459±0.025[c]
400	1.374±0.103[c]	3.597±0.191[c]	0.875±0.040[c]	0.418±0.018[c]	0.469±0.034[c]
500	1.341±0.117[c]	3.718±0.149[c]	0.853±0.118[c]	0.374±0.016[c]	0.387±0.033[c]

ChE activity determined 15 min after physostigmine administration
[a]=$p < 0.05$; [b]=$p < 0.01$; [c]=$p < 0.001$, as compared to control group.
[d]=$p < 0.05$, as compared to 200 ug/kg of physostigmine.
[e]=$p < 0.01$, as compared to 150 ug/kg of physostigmine.

Brain:

A dose of 25 ug/kg of Phy did not produce any significant change in ChE activity of brain. A dose-dependent inhibition of ChE activity was observed with 50-150 ug/kg of Phy (Fig. 2). The percentage ChE inhibition was 23%, 61% and 66% with 50, 100 and 150 ug/kg of Phy, respectively. Higher dosages of Phy did not increase the % inhibition of ChE; however, a decrease in % ChE inhibition was observed with increase in dosage (Fig. 2). The % ChE inhibition was 55%, 49%, 50% and 48% with 200, 300, 400 and 500 ug/kg of Phy, respectively. Even though there was constant increase in Phy concentration with increase in dose (Fig. 2), ChE inhibition plateaued between 200 to 500 ug/kg.

Heart:

Phy in a dose of 25 ug/kg produced a slight increase (16%) in heart ChE activity, which was statistically insignificant. Phy produced a dose-related inhibition of heart ChE; the inhibition was 16%, 27%, 31%, 38%, 47%, 49% and 50% with 50, 100, 150, 200, 300, 400 and 500 ug/kg of Phy, respectively (Fig. 3). Even though there was constant increase in Phy concentration with increase in dose (Fig. 3), ChE inhibition plateaued between 200 and 500 ug/kg.

Thigh muscle:

Thigh muscle ChE inhibition was 4%, 9%, 40%, 42%, 42%, 44%, 43% and 53% with 25, 50, 100, 150, 200, 300, 400 and 500 ug/kg Phy. However, the inhibition produced with 25 and 50 ug/kg of Phy was statistically insignificant. The

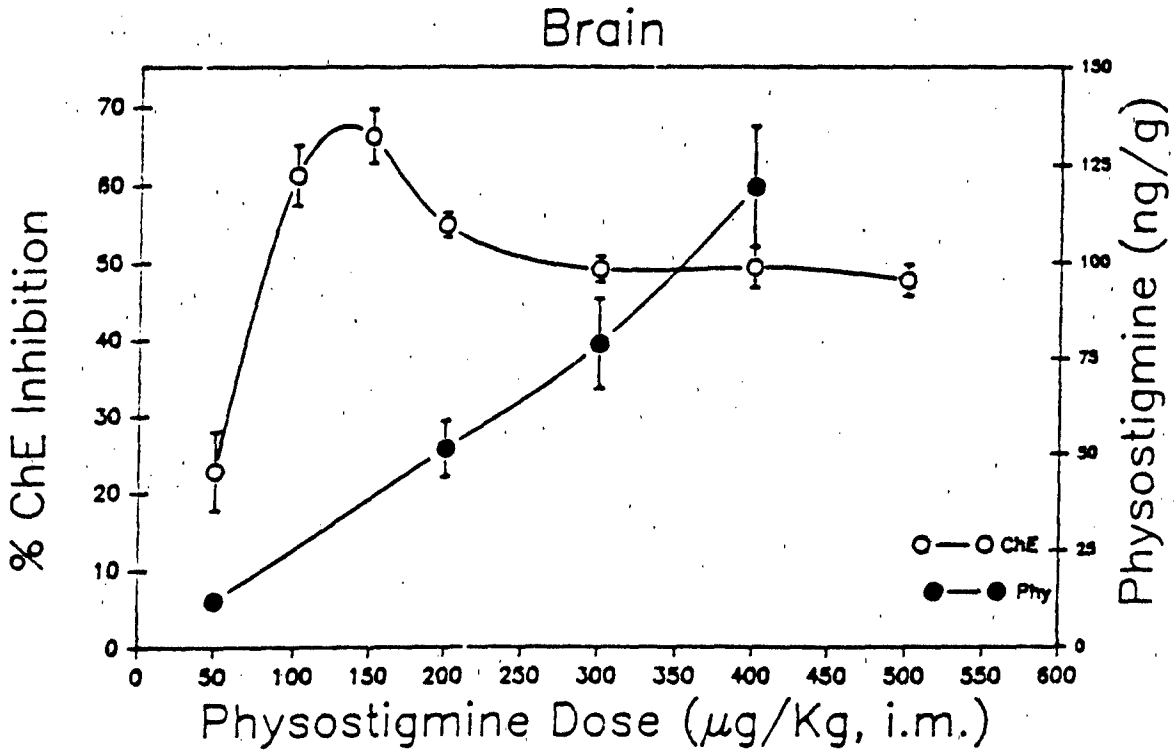

Figure 2: Relation between physostigmine concentration (●——●) and % ChE inhibition (○——○) in brain of rats administered 25-500 ug/kg of physostigmine i.m. Each point is the mean ± S.E.M. of 6-8 rats.

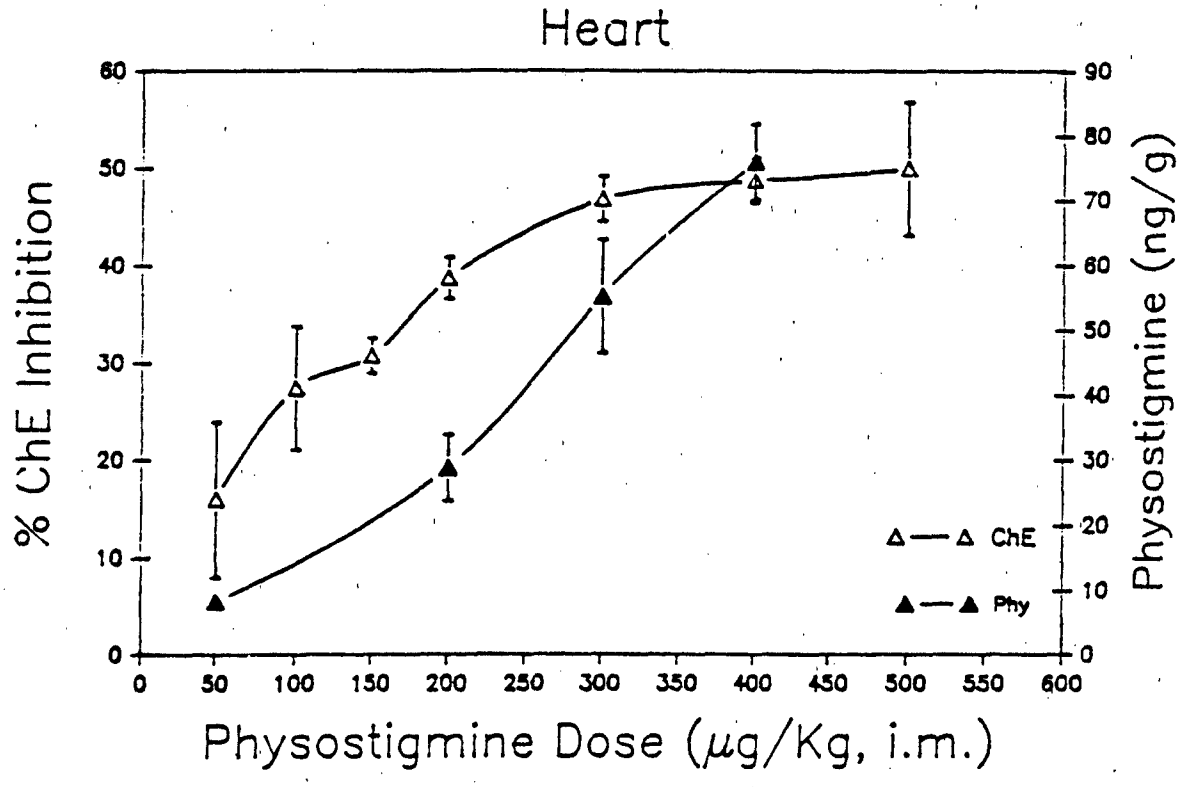

Figure 3: Relation between physostigmine concentration (▲——▲) and % ChE inhibition (△——△) in heart of rats administered 25-500 ug/kg of physostigmine i.m. Each point is the mean ± S.E.M. of 6-8 rats.

ug/kg to a 500 ug/kg dose (Fig. 4). In spite of the constant increase in the concentration of Phy with increase in the dose (Fig. 4), the ChE inhibition remained plateaued at 150-400 ug/kg then again increased at 500 ug/kg.

Discussion:

ChE inhibition is an important parameter in monitoring the efficacy of Phy in organophosphate intoxication or in Alzheimer patients. Phy has a narrow therapeutic range and the toxic dose of Phy appears to be very close to its optimum therapeutic dose of 0.25-1 mg, i.v. infusion for 30 min (64), or 0.125-0.5 mg, i.v. infusion for 30 min (65). A slight increase in dose of Phy can lead to adverse effects such as nausea, vomiting, hypersalivation and diarrhea. An excessive dose of Phy may produce severe toxic cholinergic manifestations leading to central and peripheral respiratory paralysis.

There appears to be a controversy regarding what constitutes an appropriate dose of and route of administration for Phy. Therefore, a dose response curve of Phy for ChE inhibition would be useful in selecting the optimum dose and route of administration. Our results clearly show that Phy produced a dose-dependent inhibition of RBC ChE from 25-200 ug/kg doses. However, with the higher doses, the percentage inhibition plateaued, indicating the optimal dose of Phy for ChE inhibition in RBC to be about 150-200 ug/kg in rat at 15 min after its i.m. administration. Phy concentration at this dose in plasma was 42.05 ng/ml. However, recently, Maxwell et al. (60) showed 75% ChE inhibition in whole blood with 200 ug/kg of Phy after i.m. administration at 25 min. Harris et al. (8) showed 58% ChE inhibition in whole blood at 15 min with 70 ug/kg, i.m. dose of Phy to rat. These authors have determined the total ChE present in whole blood, which comprises true ChE (AChE) and "pseudocholinesterase", i.e., butylcholinesterase (BuChE). It is understandable that our value of ChE inhibition is lower because we have estimated ChE values in RBC rather than in whole blood. Heyl et al. (5) showed similar ChE inhibition (70%) at 15 min after 250 ug/kg of Phy i.m. to rats.

Our results show that the maximum ChE inhibition in brain and diaphragm was at about 200 ug/kg and then, with an increase in dose, there was a slight but definite decrease in inhibition of ChE. This slight decrease in inhibition of AChE in RBC, brain and diaphragm may be related to the rates of carbamylation and decarbamylation of ChE of low and high molecular weights and the concentration of Phy in these tissues, which is dose-dependent. Somani and Khalique (10) have reported the rate of recovery of ChE activity at 0.027 min^{-1} in brain and .083 min^{-1} in muscle after 100 ug/kg i.v. administration of Phy. In our laboratory we have shown that the rate of recovery of ChE in diaphragm is 0.05 min^{-1} after i.m. administration of Phy; however, after oral administration, the rate of recovery is biphasic (0.053 and 0.017 min^{-1}), indicating the different rates of decarbamylation. The results in this study also show that the ChE inhibition can be predicted from Phy concentration up to 150 ug/kg dose since there is a linear correlation between ChE inhibition and Phy concentration from 50 to 150 ug/kg in brain. However, in heart, the linearity of ChE inhibition is from 50 to 300 g/kg dose, and in muscle, the linearity appears to be in small range, i.e. from 25 to 100 g/kg dose.

Phy concentration is lowest in heart and highest in brain, followed by plasma and muscle. It seems that there is a rapid equilibration between blood and brain and that Phy concentration proportionately increases in brain for all doses. The pKa of Phy is 7.9, and it is probably sequestered in the brain since it is a tertiary amine and a very lipid-soluble drug. Because of its use for ready penetration into the brain, the drug has a greater potential as a

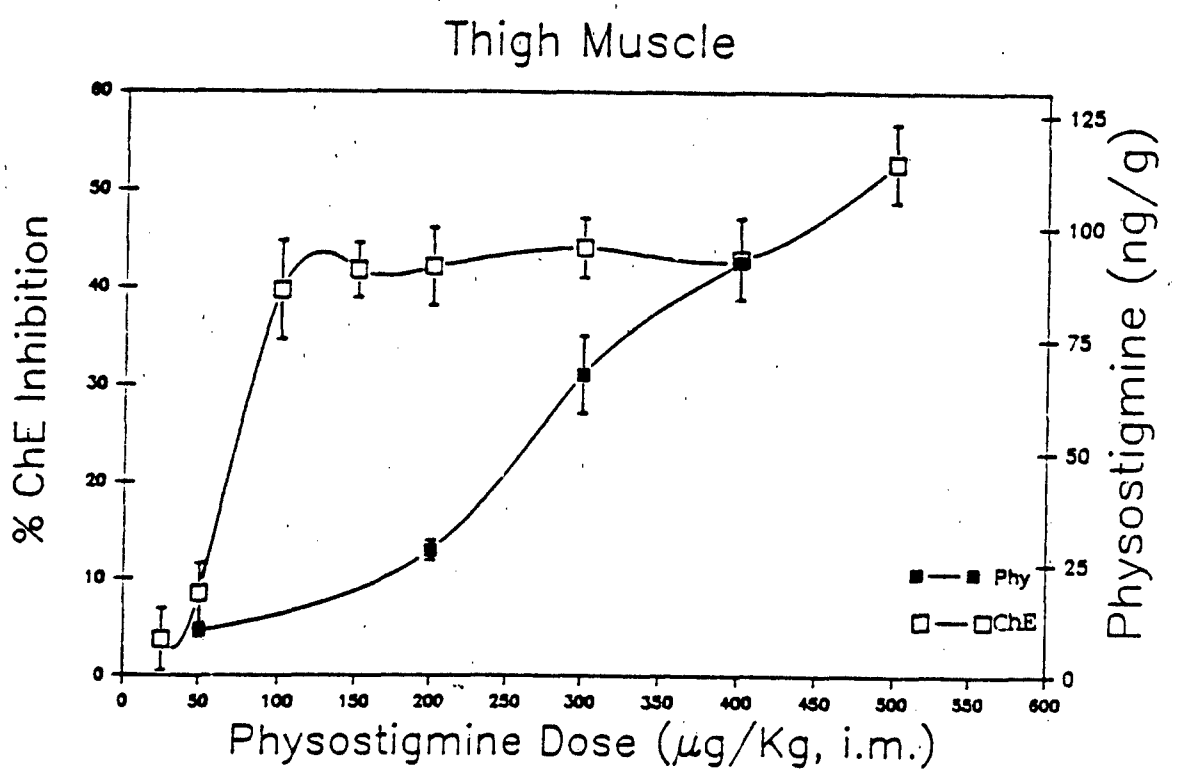

Figure 4: Relation between physostigmine concentration (■—■) and % ChE inhibition (□–□) in thigh muscle of rats administered with 25-500 ug/kg of physostigmine i.m. Each point is the mean ± S.E.M. of 6-8 rats.

pretreatment agent against organophosphate intoxication, particularly in the central nervous system. Recently, Deshpande et al. (61) have compared the efficacy of Phy (centrally acting tertiary amine) with that of pyridostigmine and neostigmine (peripherally acting quaternary amines). Pretreatment of rats with Phy 30 min prior to challenge of sarin reduced mortality from 100% to 28%. However, atropine, pyridostigmine and neostigmine injected alone did not protect rats against lethal effects of sarin. Deshpande et al. (61) have indicated that effective protection against lethality of sarin is due to protection of brain ChE by Phy. Our results (unpublished work) also show that the pretreatment of rat by Phy before administration of soman prevented the ChE enzyme from binding to soman.

Maximum ChE inhibition in brain was achieved by 150 ug/kg; then higher doses decreased ChE inhibition, which indicates that there is a threshold of Phy in rat brain. It may also be speculated that a similar threshold for Phy exists for humans, but no such study has been carried out in man. Our study suggests that the higher doses in human might be ineffective in inhibiting ChE and may produce many more side effects.

The maximum sign-free dose of Phy has been determined (2,3) by observing the effects of graded doses of Phy on the occurrence of signs of anticholinesterase poisoning (tremors, muscular fasciculations, unsteadiness, incoordination or salivation). The maximum sign-free dose of Phy without atropine was 0.16 mg/kg in chicken and guinea pig; 0.1 mg/kg in mouse, rabbit and rat; and < 0.1 mg/kg in dog. However, the maximum sign-free doses of Phy with atropine dosage (17.4 mg/kg) were 0.63, 0.3, 0.25 and < 0.1 mg/kg in guinea pig, rat, rabbit and mouse, respectively (3). Doses of Phy, when administered without atropine, were very similar in all species; however, when Phy was administered with atropine, there was greater variation for the maximum sign-free dose of Phy for the four species tested.

In conclusion, RBC, brain and diaphragm showed dose-dependent ChE inhibition over a limited dose range, whereas heart and muscle showed dose related ChE inhibition at all dosages studied. Phy concentration in plasma and tissues increased linearly with increase in dose.

II. OXYGEN CONSUMPTION, RER AND HEAT PRODUCTION IN YOUNG AND ADULT RATS AT DIFFERENT EXERCISE LEVELS.

Introduction:

Several investigators have studied the respiratory capacity of rats using various methodical approaches (66-69). None of the investigators have reported the relationship between VO_2, respiratory exchange ratio (RER) and heat production. Nor has the respiratory capacity been determined in younger rats weighing less than 200 g.

Open circuit, indirect calorimetry has been used extensively to estimate the metabolic rates of humans and large experimental animals. Systems for the determination of oxygen consumption (VO_2) in small animals have been suggested (67,69,70,72), but these systems have been limited by their inability to determine carbon dioxide production (VCO_2) and their lack of applicability in conjunction with systems where external work rate could be quantified.

There is a paucity of data regarding metabolic rates in young Sprague-Dawley rats of less than 200 g at different exercise levels. The present investigation compares responses of young rats to those of mature rats subjected to an identical exercise protocol. Specifically, the variables that were examined were the oxygen consumption, RER and heat production.

Methods:

Animals:

Two groups of male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN), young (wt. 147 $\pm$ 2 g, about 1 month) and adult (wt. 332 $\pm$ 7 g, about 4 months) were studied for the determination of metabolic variables.

Instrumentation:

The Oxyscan System and Omni-Pacer treadmill (Omnitech Inc., Columbus, OH) were used for this study, and their diagrammatic sketch is shown in Fig. 5. The Oxyscan System consists of a multi-channel flow controller, thermal mass flow meter, Oxygen Analyzer (Zirconia Sensor), Carbon Dioxide Analyzer (NDIR Sensor), and an Oxyscan Analyzer/Computer.

The flow controller consists of five input ports (marked 0, 1, 2, 3, 4). Port 0 is the reference input, while ports 1 through 4 are connected to animal chambers 1 through 4, respectively. Associated with each port is a vacuum pump and a flow-control valve. The flow controller included a mass-flow meter with a digital flow indicator. Ambient air was drawn into each metabolic chamber through a Drierite column at an approximate rate of 3650 ml/min Standard Temperature and Pressure (STP). Good quality Drierite (anhydrous $CaSO_4$, W.A. Hammond Drierite Co., Xenia, OH) was used to remove moisture from the atmospheric air as well as from the expired air.

The oxygen and CO_2 sensors were calibrated using room air, 20.2% O_2/balance N_2, 99.99% N_2 and 0.5% CO_2/balance N_2 standard gases. The calibration of the sensors was carried out every day prior to exercising the rats.

The Omni-Pacer treadmill is a compact, table-top model, with an endless conveyor belt on top of which is a plastic housing divided into four channels so that four rats can exercise at a time. The treadmill includes speed- and

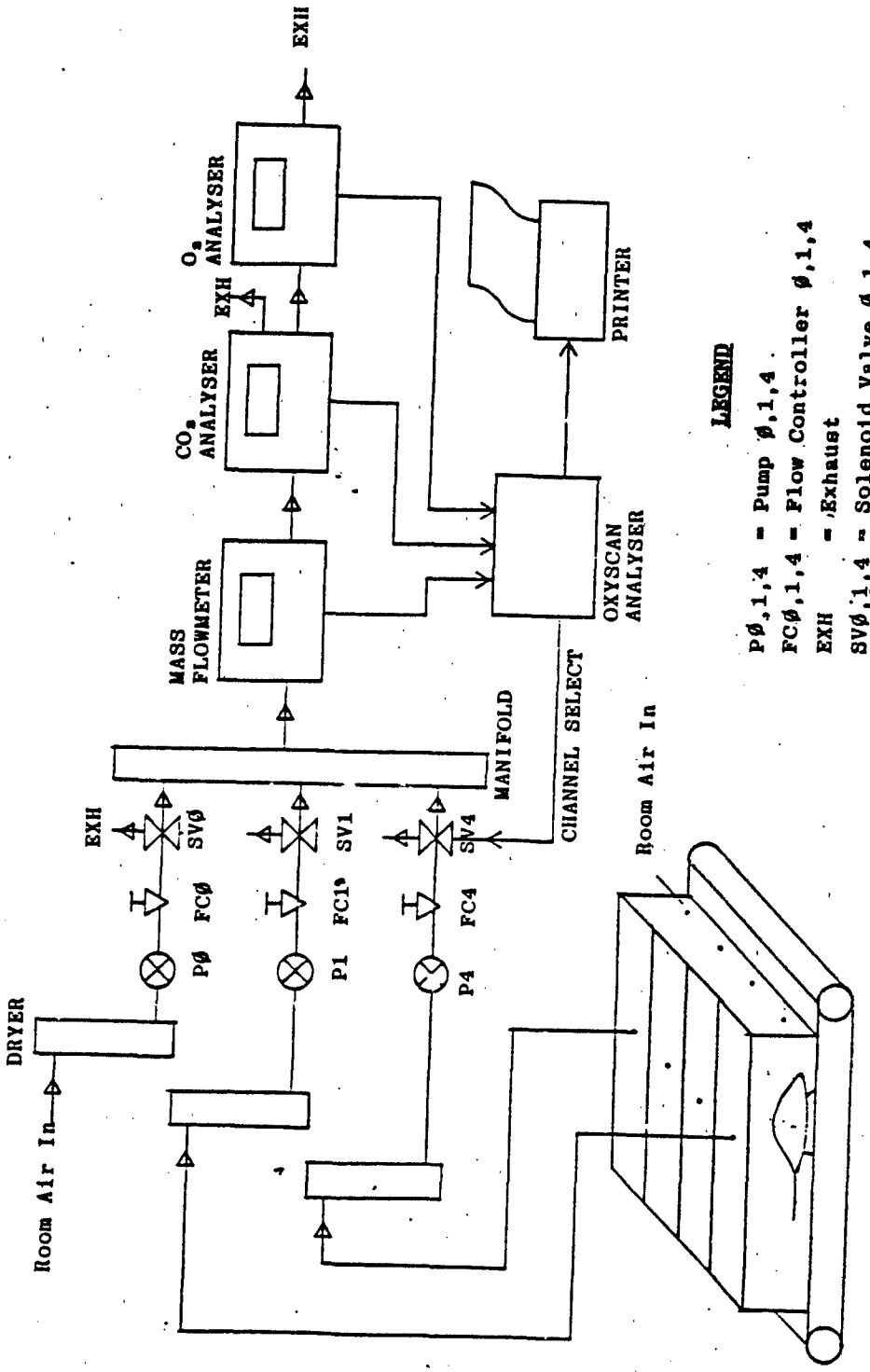

Fig. 5. Flow diagram of Oxyscan System and Treadmill

acceleration-control electronics, grade control (-25° to +25°), a shock grid and an exercise-duration timer.

Test Procedure:

The weight of each rat was recorded and four rats were exercised at a time on four different channels of the treadmill for 5 min during each level of exercise (Table 2). These four channels recorded the metabolic variables (oxygen consumption, RER and heat production) for each rat.

Table 2: Protocol for exercising rats on treadmill, at different grades and speed for constant duration.

Stage	Grade/Degrees	Speed (m/min)	Duration (min)
0[a]	0	2	5
1	0	8.2	5
2	5	15.2	5
3	10	19.3	5
4	10	26.8	5
5	12.5	26.8	5
6	12.5	30.3	5
0[b]	0	2	5

[a] Resting
[b] 10 min post-exercise

Initially the treadmill/Oxyscan System was operated without rats to determine whether there was any change in O_2 and CO_2 percentage for the rat chamber as compared to atmospheric O_2 and CO_2. (The percent change in these two parameters should be close to zero.)[2] The rats were then placed in the treadmill chamber and the percent change in O_2 and CO_2 was determined at 2.5-min intervals. Initially the animals were hyperactive in the treadmill, resulting in high RER values. Hence, the rats were acclimatized to the treadmill: they were walked at 2m/min followed by 10 m/min and then 2 m/min again for a period of 5 min at each speed to obtain consistent metabolic values. After two steady-state values in metabolic variables were observed, the rats were tested using the incremental exercise protocol as given in Table 2.

Measurements of maximal oxygen consumption (VO_2 max) were considered valid only if the animal ran until it could no longer maintain pace with the treadmill and attained an RER value of approximately 1.0. Some of the animals in the adult group which ran erratically or did not run at all at high treadmill speeds were discarded from the experiment.

The Omni-Pacer treadmill and the Oxyscan System together form an integrated system for monitoring O_2 consumption (VO_2, ml/kg/min), CO_2 production (VCO_2, ml/kg/min), respiratory exchange ratio (RER = VCO_2/VO_2) and heat

production (cal/hr). These parameters were continuously recorded and printed at intervals of 2.5 min for each of four animals at different exercise levels.

The Oxyscan System automatically monitored the O_2 concentration, CO_2 concentration and standard temperature and pressure (STP) mass flow for room air and air drawn from each animal chamber. From these basic data it determines VO_2, VCO_2, RER and HEAT using the following equations:

$$VO_2 \text{ (ml/kg/min)} = \frac{F}{W \times 100} \left[- \#X - \frac{X1}{Z1} (\#X + \#Y) \right]$$

$$VCO_2 \text{ (ml/kg/min)} = \frac{F}{W \times 100} \left[\#Y + \frac{Y1}{Z1} (\#X + \#Y) \right]$$

$$RER = \frac{VCO_2}{VO_2}$$

$$HEAT \text{ (cal/HR)} = [4.33 + 0.67 \text{ RER}] VO_2 \times W$$

Where X1, Y1, Z1 are % concentrations of O_2, CO_2 and N_2, respectively, in air.
#X, #Y are % changes in concentrations of O_2 and CO_2, respectively.
F is STPD mass flow from the animal chamber in ml/min.
W is the weight of an animal in kg entered via switches at beginning of experiment.

Statistical Analysis:

Statistical analyses were performed using a two factor split-plot analysis of variance. The between-subjects factor was a group consisting of two levels, young and adult. The within-subjects factor was stage, consisting of resting, stage 1, stage 2, stage 3, stage 4, stage 5 and stage 6. The linear increase over stage was evaluated by examining the linear components obtained from the split-plot analysis. Additional split-plot analyses were performed comparing resting with 10 min post-exercise measures. Follow-up tests were conducted using the Games-Howell procedure. Independent t-tests were used to examine VO_2, RER, and heat at their maximums. Statistical significance was set at the 0.05 level.

In an effort to examine the influence of decreasing sample size with increasing stage, split-plot analyses were performed three times for each variable (VO_2, RER, Heat). The first analysis utilized complete data (resting through stage 4) on all subjects. The next analysis contained a smaller sample of subjects, resting through stage 5. The third analysis contained the smallest sample, resting through stage 6. These successive analyses yielded similar results across tests by variable. Therefore, the following split-plot significance tests will be reported using the results from the complete data.

Results:

Young rats had a significantly higher VO_2 exercise profile ($F(1,59) = 79.12$; $p < 0.001$) than did the adult rats (Fig. 6). In addition, both the young and adult rats showed a linear increase in VO_2 with successive levels of exercise (stage). The VO_2, 10 min-post exercise analysis indicated a significant ($F(1,44) = 140.03$; $p < 0.001$) group effect, where young rats had larger means than adult rats (Fig. 6). VO_2 returned to resting values at 10 min post exercise. The young rats attained a significantly ($t(59) = 5.28$; $p < 0.001$)

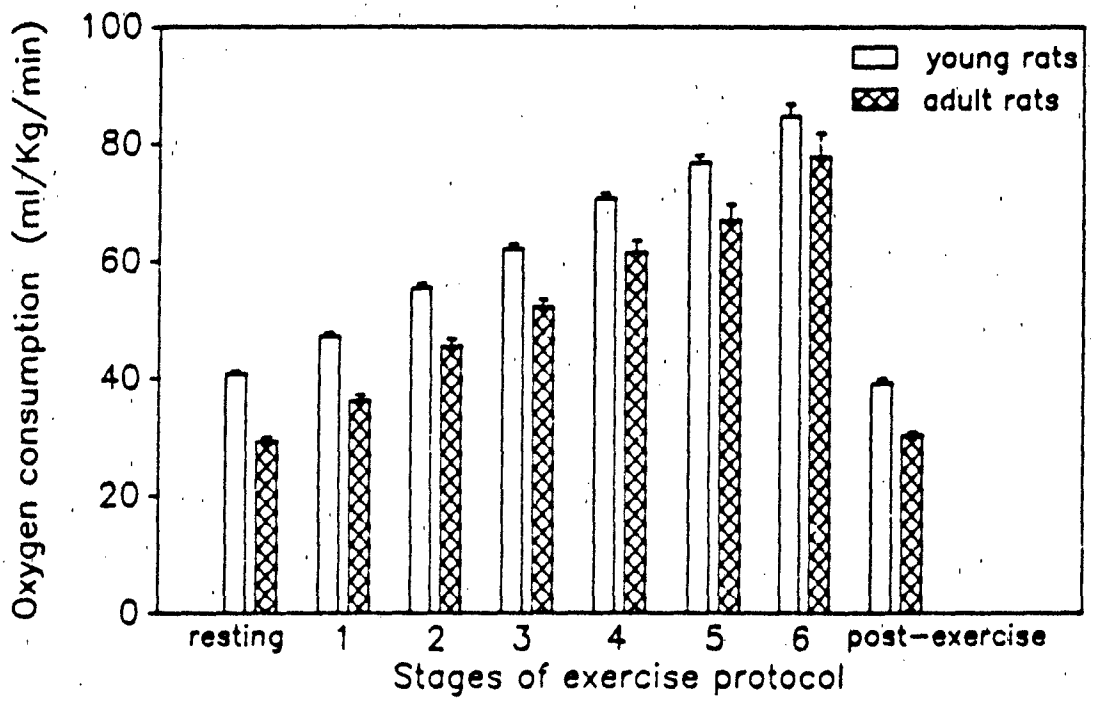

Fig. 6. Effect of different levels of exercise on Oxygen Consumption in young (n = 31) and adult (n = 30) rats. The values are Mean ± S.E.M.

higher VO_2 max (81.56 ml/kg/min) than the adult rats (69.98 ml/kg/min) despite being tested at an identical level of exercise.

The difference in the VO_2 from one level of exercise to another varied from 6.15 to 8.22 ml/kg/min and from 5.45 to 10.86 ml/kg/min in young and adult rats, respectively. The percent increase in VO_2 from one level of exercise to the subsequent stage varied from 15.05% to 21.1% and 18.65 to 37.15% in young and adult rats, respectively (Table 3).

Table 3: A comparison of VO_2, RER and heat production in young and adult rats expressed as percentage of resting.

	% Resting VO_2		% Resting RER		% Resting Heat	
Levels of Exercise	Young Rats	Adult Rats	Young Rats	Adult Rats	Young Rats	Adult Rats
Stage 1	115.59	123.71	100.51	105.24	116.18	124.32
Stage 2	135.75	155.39	104.07	109.74	136.79	157.17
Stage 3	152.09	178.42	107.12	108.77	153.16	180.18
Stage 4	173.19	210.29	110.93	115.47	176.02	214.05
Stage 5	188.24	228.94	111.05	117.29	192.35	230.68
Stage 6	207.72	266.09	111.69	115.35	211.45	271.63
Mean VO_2 Max	199.88	235.96	113.21	116.69	203.64	240.42
10 Min Post Exercise	95.98	103.49	97.59	91.96	96.62	102.51

Adult rats were unable to run at higher speeds and higher grades of exercise; only 13.33% of adult rats were able to run at Stage 6. However, 38.71% of the young rats were able to run at Stage 6 of exercise protocol.

The results of heat production, on a weight basis, were similar to the results for VO_2. That is, young rats had a significantly higher heat production profile ($F(1,59) = 69.60$; $p < 0.001$) than the adult rats (Fig. 7). Again, both young and adult rats showed a linear increase between heat production and successive levels of exercise. The heat production, 10-min-post-exercise analysis indicated a significant ($F(1,44) = 144.31$; $p < 0.001$) group effect, where young rats had larger means than adult rats (Fig. 7). Heat production returned to resting values at 10 min post-exercise. The young rats attained a significantly ($t(59) = 4.50$; $p < 0.001$) higher maximum heat production (24.11 kcal/kg/hr) than the adult rats (20.57 kcal/kg/hr) at maximal exercise.

The adult rats had a higher percentage of resting heat production (240.4%) than young rats did (203.6%) at maximal exercise (Table 3).

The results for RER are more complex than those for VO_2 and heat, because the split-plot analysis indicated a significant group by stage interaction ($F(4.236) = 4.91$; $p < 0.01$). This significant interaction effect indicates that the profiles for young and adult rats differ across stages, as shown in Figure 8. Although both the young and adult rats exhibit increases in RER with successive levels of exercise, the increases are no longer parallel. The Games-

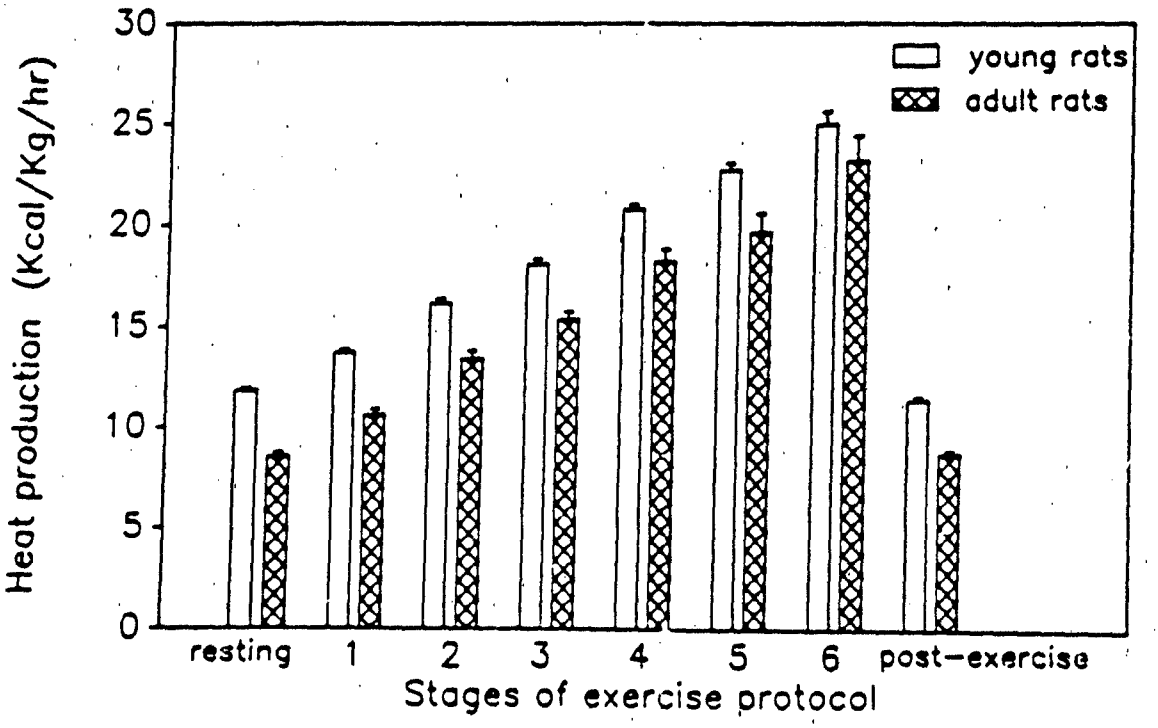

Fig. 7. Effect of different levels of exercise on heat production in young (n = 31) and adult (n = 30) rats. The values are Mean ± S.E.M.

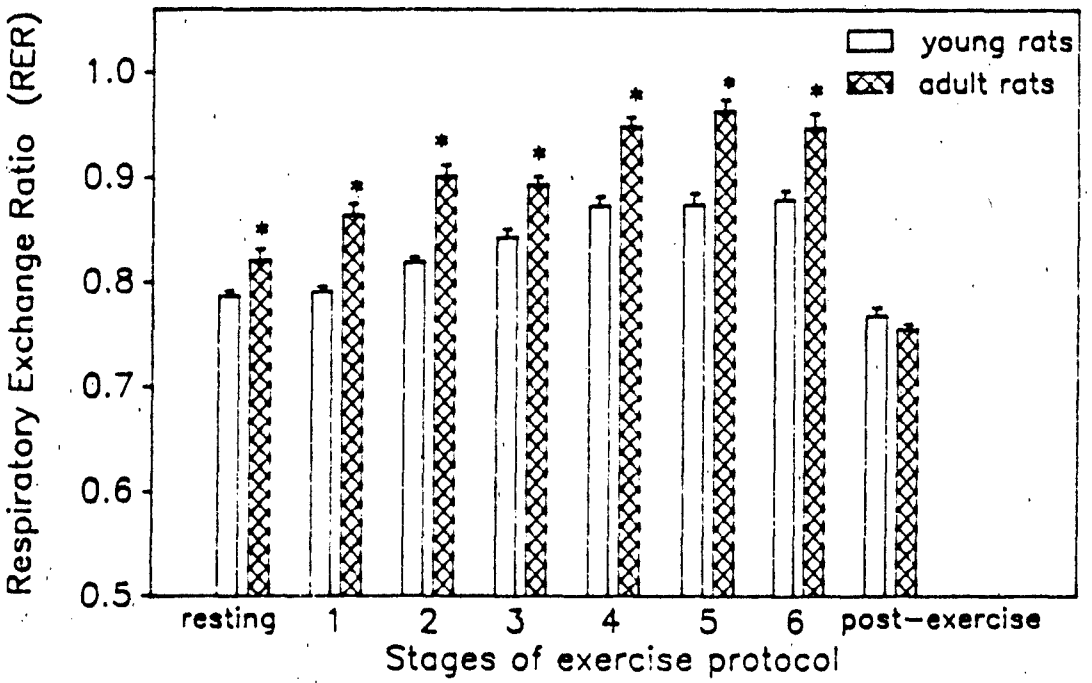

Fig. 8. Effect of different levels of exercise on Respiratory Exchange Ratio in young (n = 31) and adult (n = 30) rats. The values are Mean ± S.E.M.

Howell follow-up tests indicate that adjacent means, especially in the adult group, fluctuate between increasing and decreasing trends. In addition, the follow-up tests indicate that the adult group had significantly higher means than the young group at every stage (Fig. 8). The RER, 10-min-post-exercise analysis also indicates a significant interaction between group and stage ($F(1,44) = 13.26$; $p < 0.001$), where stage consists of resting and 10 min post-exercise. Examination of the means indicates that adult rats had a higher RER mean than the young rats at resting, but by post exercise adult rats had attained a lower mean than the young rats (Fig. 8).

In comparison to resting values, the percentage increases in RER to VO_2 max were 13.21% in young and 16.69% in adult rats (Table 3). The 10-min-post-exercise values of R R were similar to resting RER in both young and adult rats.

Discussion:

The present investigation indicates that an age-related decline in VO_2 is evident at an early age in Sprague-Dawley rats, i.e., 1 month to 4 months of age. This decline is important when attempts are made to identify optimal levels of other variables. An age-related difference in VO_2 has also been observed in 4-month (young) vs. 18-month (old) rats (73). Although our study and Cartee and Farrar's study (73) investigated 4-month old rats, a direct comparison cannot be made since the weight of the rats was not mentioned in Cartee and Farrar's study (73).

The maximum oxygen consumption (VO_2 max) is considered to be the best single measure of aerobic capacity in man and animals and may be affected by a number of factors. The adult rats (68.97 ml/kg/min) have shown a lower VO_2 max than the younger rats (81.55 ml/kg/min). Our findings are in agreement with those of Cartee and Farrar (73), who have also reported a difference in VO_2 max of young and adult rats. However, there was a quantitative difference between their values and our values. This difference may be due to sex, strain, age, or weight of the animals. The lower VO_2 max found in adult rats may also be attributed to decreased physical activity (74). Old rats have a lower hindlimb muscle respiratory capacity than young rats (75,76). These differences, however, might not be the result of aging per se. It is likely that the documented decline in spontaneous physical activity in old rats (77-81) contributes to the age-related change. Recently, Musch et al., (82) have also shown a VO_2 max of 80 ml/kg/min in rats weighing 300 g; however, the method of measurement of VO_2 max was different than our method. The results indicate that the adult rats had to consume a greater percentage of initial oxygen throughout the incremental exercise protocol than young rats did, though both groups of rats were given identical exercise.

RER values (Fig. 8) indicated that carbohydrates were being utilized more readily in adult rats toward the end of exercise than in younger rats. The young rats (0.787 ± 0.005) had significantly lower resting RER than did adult rats (0.821 ± 0.011). This indicates a greater dependence on fat metabolism in young than in adult rats. We could not observe RER > 0.9 except in a few rats at VO_2 max. Our data for RER are in agreement with those of Bedford et al., (83) and Brooks and White (66). However, Cartee and Farrar (73) observed an RER of 1.12 to 1.16 at VO_2 max, but they used trained rats. It is possible that trained rats are able to maintain a greater percentage of carbohydrate use at higher intensity of exercise than are our untrained rats.

The young rats showed less heat production than the adult rats did. However, on a unit mass basis (per kg body weight), young rats produced more

heat than the adult ones. This is due to the fact that small rats possess a higher metabolic rate.

Body weight is an important factor that affects energy expenditure in many forms of exercise. Taylor et al., (69) have shown that the cost of running, expressed as the oxygen needed to transport 1 kg of body weight over 1 km, decreases regularly with increasing body size in various mammals. It is possible that body weight plays a more significant role in determining VO_2 max in untrained rats than age of the animal does. This factor supports our data in correlating the body weight to VO_2 max.

In conclusion, the results have indicated significant differences in oxygen consumption and heat production in young vs. adult rats undergoing identical types of exercise. The young rats attained a higher VO_2 max (81.55 ml/kg/min) than the adult rats did (68.97 ml/kg/min). The younger rats possessed a higher resting heat capacity (11.84 kcal/kg/hr) than the adult rats did (8.53 kcal/kg/hr). It appears that the body weight has a significant effect in determining the VO_2 max in untrained Sprague-Dawley rats regardless of their age.

III. EFFECTS OF PHYSOSTIGMINE AND DIFFERENT LEVELS OF CONCURRENT ACUTE EXERCISE ON THE CHOLINESTERASE ACTIVITY IN RBC AND TISSUES OF RATS.

Introduction:

Physical exercise is one of the important factors that alter ChE enzymatic activity (84,85). The intensity of changes depends upon the type and severity of exercise (86,87).

Phy is a centrally acting anticholinesterase drug and easily crosses the blood-brain barrier (10). Phy is considered to be a potential pretreatment agent against organophosphate poisoning. McMaster and Carney (88) have demonstrated that acute exercise increases behavioral sensitivity to Phy. Carney et al. (89) have also reported exercise-induced changes in CNS-acting anticholinergic drug potency. Pyridostigmine bromide, another anticholinesterase drug, has been reported to produce a variety of debilitating effects during exercise in the heat in rats (14). The commonly held belief that physical fitness level and mental health are positively correlated (90) implies an exercise-induced alteration in brain function. If some change in brain function does occur due to exercise, then such an alteration should produce measurable behavioral changes in human and non-human subjects.

Phy at a dose of 200 ug/kg has been reported to reduce the endurance time and increase the rate of rise of core temperature in rats weighing about 500 g (91). Chronic exercise results in relatively long term biological changes in a number of systems. Acute exercise affects the same systems but in a more transient manner. The changes that occur in the cardiovascular and respiratory systems as a result of acute or chronic exercise have been the subject of many investigations. The changes that occur in cholinergic systems have not received much attention. Somani and Dube (92) have reported the in vivo dose response of Phy and ChE activity in RBC and tissues of rat. There seems to be an interaction between exercise and neurotransmitter ACh. This interaction can be monitored by the determination of AChE enzyme which hydrolyzes ACh in the body. The effects of cholinesterase inhibition due to physostigmine administration on work performance are of extreme importance.

The effect of Phy and concurrent acute exercise on ChE activity has not been known. Therefore, this work presents the interaction of Phy and different levels of treadmill exercise on ChE activity in RBC and various tissues (brain, heart, diaphragm and thigh muscle) and on endurance time in rats.

Materials and Methods:

Exercise and physostigmine administration:

The rats were exercised at different levels on a treadmill, as described in Table 2 of Section II, to obtain the VO_2 max, RER and heat production. After 3 days of determining the VO_2 max of each rat, the animals were divided into groups of four rats. The following experimental protocol was carried out: i) Groups of four rats were exercised at each level of exercise (50%, 80% and 100% VO_2 max), which corresponded to 10, 20 and 30 min, and soon after exercise, the rats were sacrificed. ii) Groups of four rats were administered physostigmine (70 ug/kg, i.m.) and were sacrificed at 10, 20 and 30 min. iii) Groups of four rats were administered Phy (70 ug/kg, i.m.) and then immediately exercised at 50%, 80% and 100% VO_2 max. Immediately after exercise, the rats were sacrificed. iv) In the control group, six rats were administered saline and sacrificed. Soon after the decapitation, blood, brain, heart, diaphragm, and

thigh muscle were collected. The blood was processed for determination of RBC-ChE. The tissues were stored at -70°C until analysis for ChE determination.

The ChE was determined in RBC, brain, heart, diaphragm and thigh muscle by the radiometric method as described in Section I. The ChE values of RBC are expressed as umol of ACh hydrolyzed/min/g of hemoglobin content, whereas the tissue ChE values are expressed as umol of ACh hydrolyzed/min/g of wet weight of tissue.

Determination of hemoglobin:

The hemoglobin content of blood was determined by Sigma diagnostic kit using a Beckman spectrophotometer at 540 nm.

Data analysis:

The ChE values were subjected to one-way analysis of variance with 10 levels followed by Duncan's multiple-range follow-up tests. Significant differences were accepted at $p < 0.05$.

Results:

Effect on endurance time:

The values for endurance time of rats at different levels of exercise with or without Phy administration are given in Table 4. The endurance times were 8.75, 16.25 and 23.13 min at 50%, 80% and 100% VO_2 max, respectively (Table 4). Physostigmine administration (70 ug/kg, i.m.) and concurrent exercise increased the endurance times to 11.25, 21.25 and 30.1 min at 50%, 80% and 100% VO_2 max, respectively. It seems that the cholinesterase inhibitors, in general, reduce endurance time; however, Phy at this dose (70 ug/kg, i.m.) significantly increased ($p < 0.01$) the endurance time at 100% VO_2 max.

Table 4. Effect of physostigmine and different levels of acute exercise on endurance time in rats.

Groups	n	Endurance Time (min) Mean + S.E.M.
VO_2 max 50%	4	8.75 ± 2.61
Phy 70 ug/kg + VO_2 max 50%	4	11.25 ± 1.61
VO_2 max 80%	4	16.25 ± 2.61
Phy 70 ug/kg + VO_2 max 80%	4	21.25 ± 1.61
VO_2 max 100%	4	23.13 ± 1.19
Phy 70 ug/kg + VO_2 max 100%	4	30.10 ± 1.02

Effect of exercise on ChE Activity in RBC:

The effects of physostigmine administration (70 ug/kg, i.m.) and different intensities of concurrent acute exercise 50%, 80% and 100% VO_2 max corresponding to 10, 20 and 30 min on ChE activity in RBC are presented in Table 5 and Fig. 9.

Significant differences in ChE activity in RBC (Table 6) were not shown at 50%, 80% and 100% VO_2 max, indicating that the different intensities of acute exercise did not have a profound effect on ChE activity. It seems from these data that the ChE has recovered to about 73-79% of control within 10-30 min after Phy administration. Administration of Phy and then concurrent exercising of rats at different intensities of VO_2 max did not alter ChE activity significantly ($p > 0.05$). However, there seems to be significant difference in ChE activity between exercise alone ($p < 0.01$), Phy alone ($p < 0.01$), and Phy + exercise ($p < 0.01$) (Table 5).

Effect of exercise on ChE activity in brain:

Different levels of exercise (50%, 80% and 100% VO_2 max) did not produce any significant effect on ChE activity (Table 5, Fig. 10). Administration of Phy significantly decreased the ChE activity (65-68% of control) in brain ($p < 0.01$) at 10, 20 and 30 min (Fig. 10). The administration of Phy and then concurrent acute exericse further decreased the ChE activity (58-44% of control) ($p < 0.01$) on all three levels of exercise.

Effect of exercise on ChE activity in heart:

Three intensities of acute exercise (50%, 80% and 100% VO_2 max) decreased the ChE activity (about 82-85% of control) (Tables 5 and 6, Fig. 11). Administration of Phy produced ChE activity that was 74% of control 10, 20 and 30 min after its administration. Phy followed by acute exercise produced ChE activity that was about 77% of control at 50% and 80% VO_2 max and did not show any significant effect as compared to Phy alone or exercise alone. The results indicate that the heart is the most susceptible organ affected by three different levels of acute exercise compared to control (Fig. 11).

Table 5. Effects of physostigmine administration (70 ug/kg, i.m.) and different levels of concurrent acute exercise (50%, 80% and 100% VO_2 max) on cholinesterase activities in RBC and tissues of rats. Values are Mean ± SEM.

Groups	Tissues umol/min/g				
	RBC umol/ min/gHb	Brain	Heart	Diaphragm	Thigh Muscle
Control	1.688 ± 0.144	8.103 ± 0.609	1.108 ± 0.124	0.723 ± 0.043	0.778 ± 0.045
VO_2 max 50%	1.962 ± 0.081	7.457 ± 0.223	0.941 ± 0.019	0.683 ± 0.014	0.695 ± 0.039
VO_2 max 80%	1.870 ± 0.041	6.886 ± 0.141	0.936 ± 0.013	0.698 ± 0.013	0.733 ± 0.039
VO_2 max 100%	1.827 ± 0.106	7.072 ± 0.181	0.914 ± 0.041	0.701 ± 0.033	0.777 ± 0.044
Phy - 10 min	1.226 ± 0.103	5.372 ± 0.608	0.809 ± 0.021	0.494 ± 0.017	0.443 ± 0.028
Phy - 20 min	1.294 ± 0.103	5.342 ± 0.369	0.814 ± 0.032	0.499 ± 0.045	0.418 ± 0.026
Phy - 30 min	1.425 ± 0.027	5.626 ± 0.219	0.815 ± 0.026	0.585 ± 0.041	0.425 ± 0.044
Phy + VO_2 max 50%	0.908 ± 0.071	4.666 ± 0.403	0.855 ± 0.011	0.498 ± 0.013	0.418 ± 0.018
Phy + VO_2 max 80%	0.802 ± 0.053	3.601 ± 0.236	0.843 ± 0.036	0.525 ± 0.069	0.488 ± 0.045
Phy + VO_2 max 100%	0.853 ± 0.071	4.068 ± 0.073	0.809 ± 0.021	0.598 ± 0.054	0.497 ± 0.040

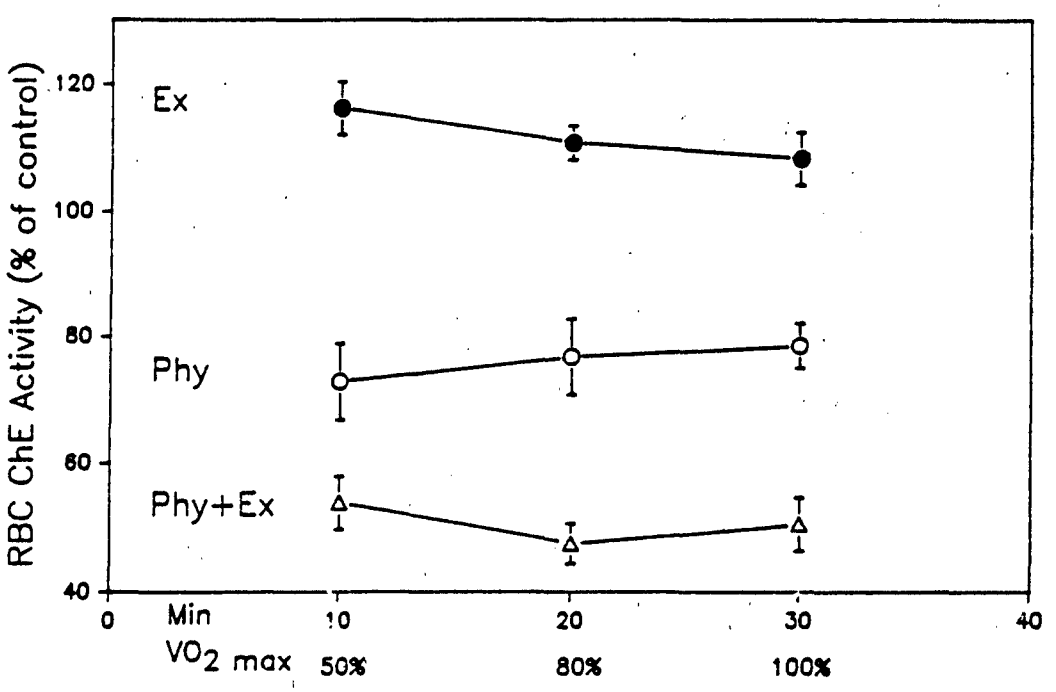

Fig. 9. Effects of physostigmine administration (70 ug/kg i.m.) and different levels of concurrent acute exercise on ChE activity of RBC in rats. Values are mean ± S.E.M.

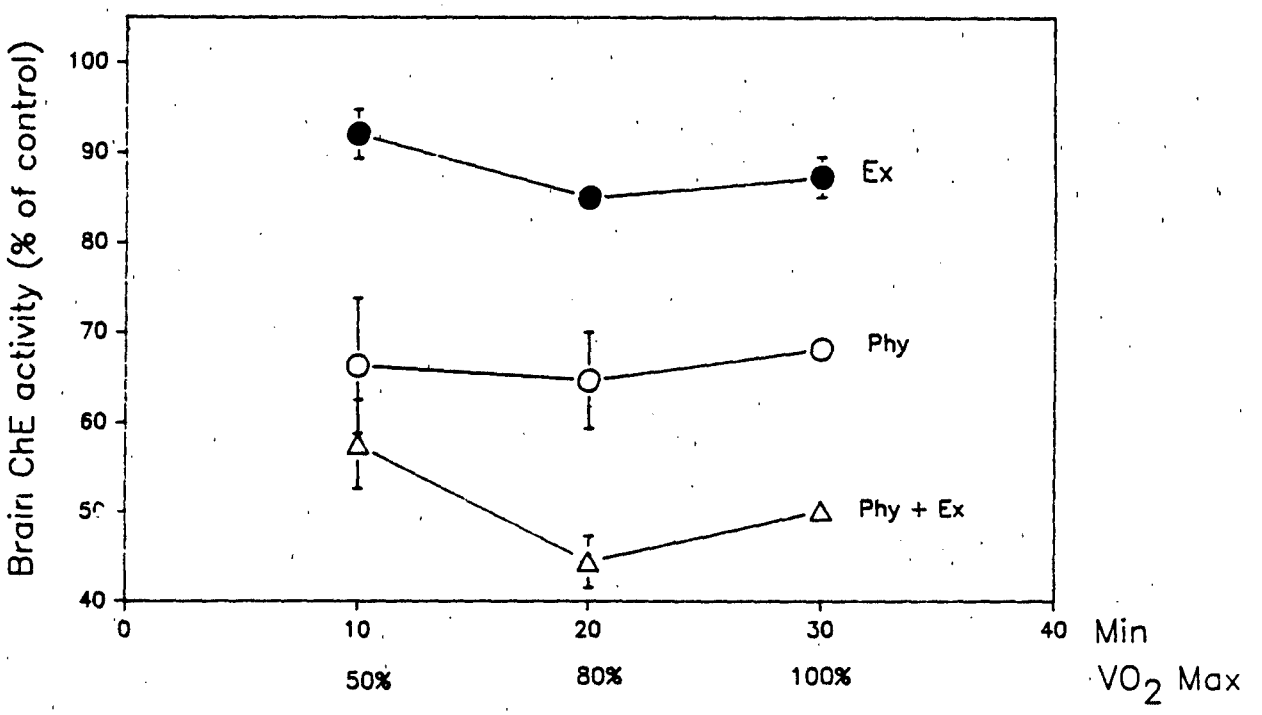

Fig. 10. Effects of physostigmine administration (70 ug/kg, i.m.) and different levels of concurrent acute exercise on ChE activity of brain in rats. Values are mean ± S.E.M.

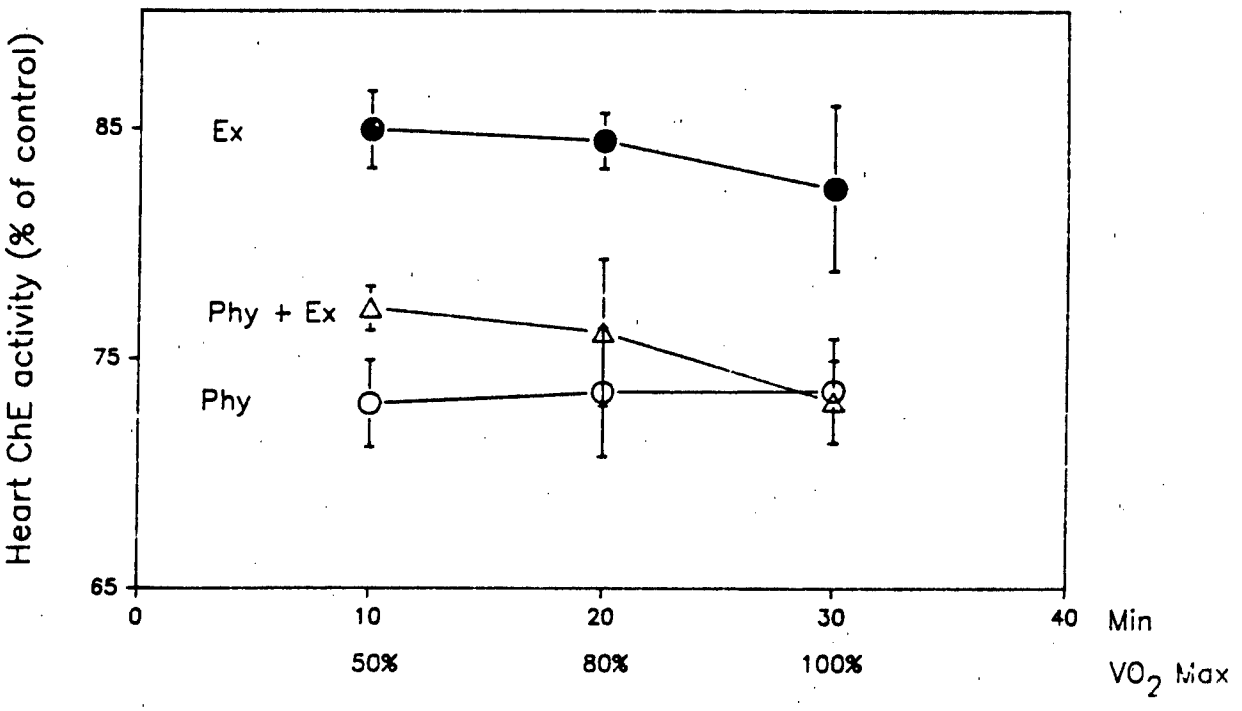

Fig. 11. Effects of physostigmine administration (70 ug/kg, i.m.) and different levels of concurrent acute exercise on ChE activity of heart in rats. Values are mean ± S.E.M.

Effect of exercise on ChE activity in diaphragm:

Different levels of acute exercise did not produce any significant effect on ChE activity (Tables 5 and 6, Fig. 12). Administration of Phy resulted in decline in ChE activity to 68, 69 and 81% of control at 10, 20 and 30 min, respectively. These results indicate that the recovery of ChE enzyme had started in diaphragm, resulting in reduced ChE inhibition (81% of control) at 30 min. The effect of Phy and concurrent acute exercise on ChE inhibition was significantly greater compared to exercise alone. The ChE activity in Phy alone as well as Phy and concurrent exercise was almost the same from 10-30 min (Fig. 12).

Effect of exercise on ChE activity in thigh muscle:

Different levels of exercise did not produce any significant effect on ChE activity of thigh muscle (Table 5, Fig. 13). However, there was a slight decrease in ChE activity: 90% and 94% of control at 50% and 80% VO_2 max. Administration of Phy showed a significant decrease in ChE activity (54-57% of control) from 10-30 min ($p < 0.01$). Phy and concurrent exercise elicited a significant decrease of ChE activity ($p < 0.01$) at all three exercise levels as compared to exercise alone. ChE activities were 54%, 63% and 64% of control at 10, 20 and 30 min, respectivly, in the rats administered Phy followed by acute exercise. However, there was no significant difference in ChE activity between Phy alone and Phy followed by acute exercise (Table 5).

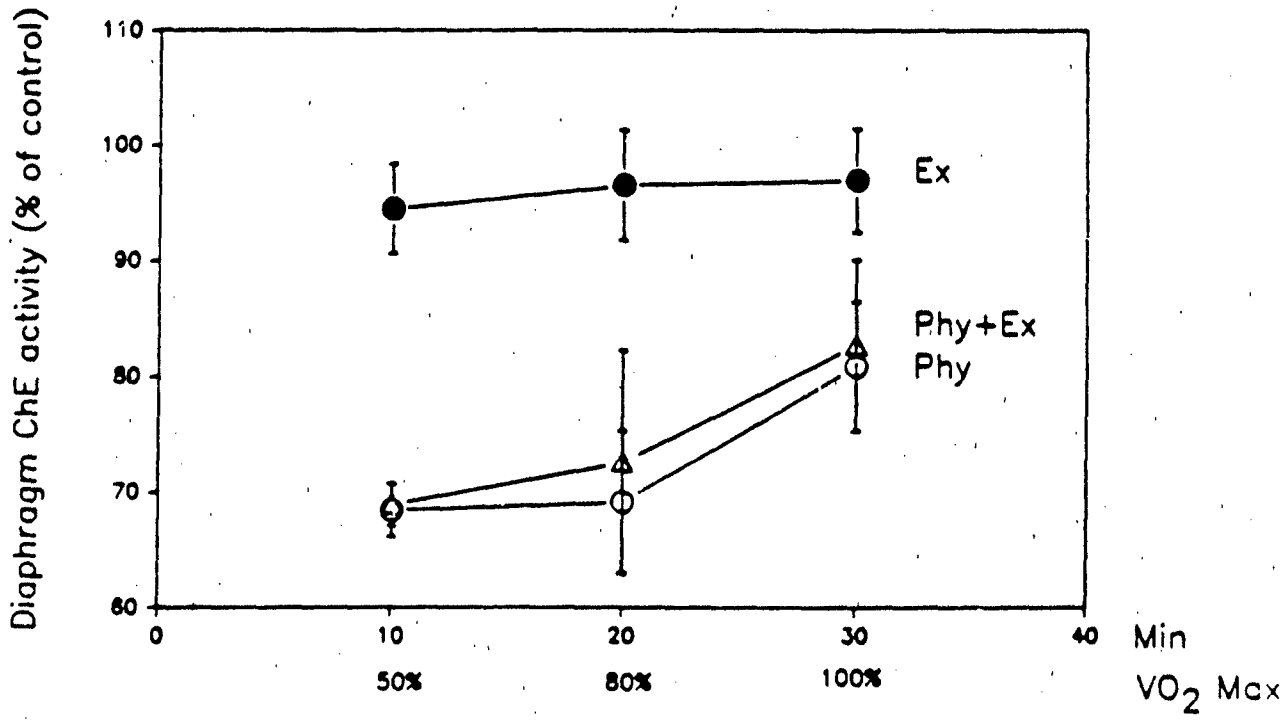

Fig. 12. Effects of physostigmine administration (70 ug/kg, i.m.) and different levels of concurrent acute exercise on ChE activity of diaphragm in rats. Values are mean ± S.E.M.

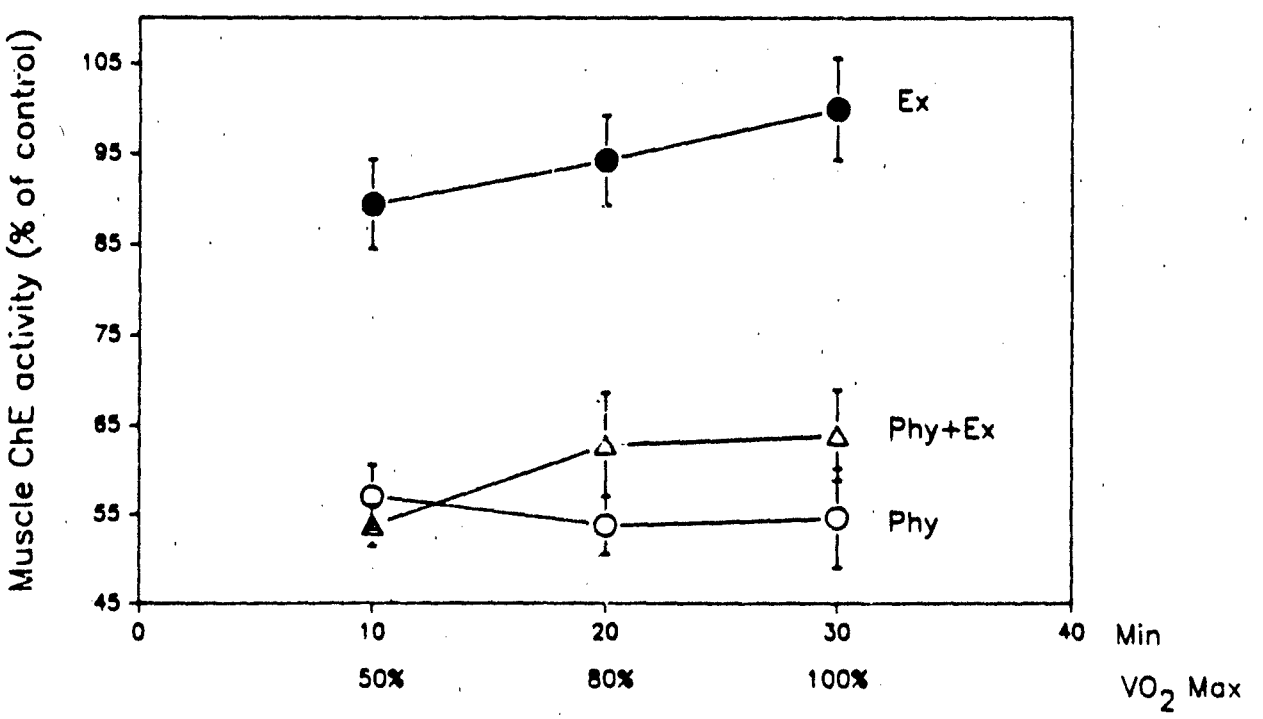

Fig. 13. Effects of physostigmine administration (70 ug/kg, i.m.) and different levels of concurrent acute exercise on ChE activity of thigh muscle in rats. Values are mean ± S.E.M.

Table 6. Comparison of p values' (* $p < 0.05$; ** $p < 0.01$ vs. control) for the data in Table 5 for ChE in tissues and RBC.

Treatment	RBC	Brain	Heart	Diaphragm	Thigh
Phy 10 min	**	**	**	**	**
Phy 20 min	*	**	**	**	**
Phy 30 min	**	**	**	**	**
VO_2 50%			*		
VO_2 80%			*		
VO_2 100%			*		
Phy + VO_2 50%	**	**	**	**	**
Phy + VO_2 80%	**	**	**	**	**
Phy + VO_2 100%	**	**	**		**

Discussion:

The results of the present investigation indicate that moderate inhibition (39%) of ChE activity in RBC resulted in significantly increased endurance time in rats. This is in direct contrast to the earlier work of Francesconi et al. (14) with pyridostigmine wherein strong ChE inhibition (64%) had significantly reduced the endurance capacity. Several workers have examined the effects of organophosphate-induced ChE inhibition on mental performance (93, 94), physiological responses (95, 96) and metabolism (97, 98). Very little is known about the effects of Phy-induced ChE inhibition in RBC and tissues of rat undergoing treadmill exercise at different intensities (50%, 80% and 100% VO_2 max).

Exercise at 50%, 80% and 100% VO_2 max (corresponding to approximately 10, 20 and 30 min, respectively) produced 110-119% of control ChE activity in RBC. Changes in ChE activity observed in our experiment agree with the earlier work of Pawlowska et al. (86), who have shown a significant increase in ChE activity in blood serum 1 and 2 hr after physical exercise (20 m/min for 30 min) in rats. Surprisingly, increase in the intensity of exercise did not change the ChE activity; rather, we observed a slight decrease in ChE activity (110% of control) at 100% VO_2 max as compared to 50% VO_2 max (119% of control). This might be due to excitement, anxiety and stress during the initial period of exercise (50% VO_2 max); after that, there may be a cholinergic acclimatization at higher intensity of exercise corresponding to treadmill exercise for 20 and 30 min. This may be supported by the findings on variations in RER during initial exercise. RBC-ChE was 73-61% of control after Phy administration. This finding is in agreement with our earlier studies (10,92). Administration of physostigmine followed immediately by acute exercise further decreased the ChE activity in RBC; 58%, 44% and 50% of control at 50%, 80% and 100% VO_2 max,

respectively, as compared to physostigmine alone. This might be due to increased blood flow during exercise (99-101) resulting in the rapid absorption and transport of physostigmine from the site of injection.

Different intensities of acute exercise produced a slight but insignificant decrease in brain ChE activity (85-92% of control). This finding is in agreement with Ryhanen et al. (85) and is contrary to the finding of Pedzikiewicz et al. (84), who have reported a slight increase (3%) in brain ChE activity after a single period of exercise. Holmstedt (102) has reported that physical exercise accelerates the nerve action in CNS, resulting in the increased amount of ACh in the nerve endings, and, hence, the increased amount of AChE in the tissues. Administration of physostigmine immediately followed by acute exercise further increased the ChE inhibition.

Different intensities of acute exercise produced a significant reduction of ChE activity in heart (82-85% of control). Our finding is contrary to the earlier study of Tipton et al. (103), who did not find any significant change in AChE activity after physical exercise during their studies on cardiac metabolism. We determined the ChE activity of whole heart, so we cannot quantitate the change in auricle or ventricle ChE activity. The results indicate that heart is the organ most greatly affected during exercise due to increase in workload. The decrease in heart ChE may result in an increased amount of ACh, thereby causing bradycardia. Physostigmine decreased ChE activity in heart (73% of control) from 10-30 min. Administration of physostigmine and concurrent acute exercise did not alter the ChE activity. Probably, the heart had already reached the threshold effect of exercise alone, and physostigmine administration and then exercise did not modify this threshold effect.

Different intensities of exercise did not change the ChE activity in diaphragm. Ryhanen et al. (85) have reported no change in ChE activity; however, they observed an increase in total ChE and BuChE in diaphragm after moderate physical exercise. Physostigmine reduced the ChE activity of diaphragm (68-81%) of control). Administration of physostigmine followed by concurrent acute exercise did not alter the ChE activity in diaphragm as compared to Phy alone; however, ChE activity was much less compared to exercise alone.

Different intensities of exercise did not significantly affect the ChE activity (90-100% of control) in muscle. Pedzikiewicz et al. (84) have reported an increase in muscle (20%) ChE activity after a short-term physical exercise. The increase in ChE activity may be due to an increase in blood flow in skeletal muscles (100, 101). However, muscarinic cholinergic receptors do not play a significant role in elevating muscle blood flow in conscious rats, either during the pre-exercise anticipatory phase or during slow locomotor exercise (104). It is well known that physical exercise evokes a number of enzymatic changes (especially in muscles and liver) and that the intensity of the enzymatic changes depends on the kind and severity of exercise (105, 106). Pedzikiewicz et al. (84) used a different exercise protocol (48 m/min) and, hence, observed an increase in muscle ChE activity. Physostigmine decreased ChE activity (54-57% of control). Administration of physostigmine and concurrent acute exercise did not alter the effect of physostigmine on ChE activity.

In conclusion, these results indicate that different intensities of acute exercise (50%, 80% and 100% $\dot{V}O_2$ max) resulted in a significant inhibition of ChE activity only in heart without significantly affecting brain, diaphragm and thigh muscle. However, acute exercise produced a slight increase in ChE activity of RBC. Phy inhibited ChE in RBC and in the tissues. Phy followed by

acute exercise further increased the ChE inhibition in RBC and brain without affecting heart, diaphragm and thigh muscle.

IV. THE EFFECT OF PHYSOSTIGMINE ADMINISTRATION AND CONCURRENT ACUTE EXERCISE ON BLOOD BIOCHEMICAL PARAMETERS IN RATS.

Introduction:

Exercise has been shown to influence the enzymatic sensitivity to a number of compounds acting primarily on the cholinergic system (88). The effects of cholinesterase inhibition on work performance are of extreme importance. Physostigmine, a short-acting anticholinesterase agent, has been reported to reduce the endurance time and to increase the rate of rise of core temperature in rats (91).

Lactate and pyruvate are two energy-rich compounds which are interconvertible depending upon the physiological state of the tissues. During prolonged anaerobic muscular exercise, pyruvate is converted into lactate quickly to provide instant energy for exercise. The levels of blood lactate and pyruvate provide an objective indication of the relative severity of exercise and may also affect the adequacy of the recovery process. Lactate and pyruvate assays are widely used as measures of oxygen debt. Hemoglobin and hematocrit values indicate the oxygen-carrying capacity of blood. They also give a fairly good indication of changes in plasma volume, plasma shift, cell volume and blood-transporting capacity.

Little information is available on the interaction of Phy administration and concurrent acute exercise on blood biochemical parameters. This section describes the effects of different levels of acute exercise and concurrent administration of physostigmine on hemoglobin, hematocrit, plasma lactate, plasma pyruvate and endurance time of rats.

Materials and Methods:

Animals:

Male Sprague-Dawley rats (Harlan Industries) weighing 160-200 g were used for the present study.

Exercising of rats in the treadmill:

This has already been described in Section III.

Exercise and physostigmine administration:

This has already been described in Section III.

Biochemical determinations:

Plasma was immediately separated from blood through centrifugation and was deproteinized with 8% perchloric acid. The supernatant was used for the estimation of lactate and pyruvate. Pyruvate and lactate were determined by an enzymatic method using a Sigma Diagnostic Kit and expressed as mg/dl.

Hemoglobin was determined from the blood by the cyanomethemoglobin method and was expressed as g/dl. Hematocrits were determined in triplicates and expressed in percents.

Results:

Plasma lactate:

Acute exercise at 50%, 80% and 100% VO_2 max produced 98%, 180% and 219% of control plasma lactate, respectively (Table 7, Fig. 14). Phy alone did not produce any significant effect on lactate concentration. Phy administration and concurrent acute exercise produced lactate concentrations of 138%, 144% and 179% of control at 50%, 80% and 100% VO_2 max, respectively (Table 7, Fig. 14). These results indicate that Phy administration significantly increased plasma lactate during concurrent exercise as compared to exercise alone at 50 and 80% VO_2 max or Phy alone.

Plasma pyruvate:

Acute exercise increased plasma pyruvate concentration to 66%, 170% and 228% of control at 50%, 80% and 100% VO_2 max, respectively. Phy administration produced a plasma pyruvate concentration of 118%, 90% and 75% of control at corresponding times of each exercise level. Phy administration and concurrent acute exercise produced plasma pyruvate concentrations of 170%, 155% and 152% of control at 50%, 80%, and 100% VO_2 max, respectively (Table 7, Fig. 15).

Hemoglobin:

Acute exercise produced a hemoglobin content of 112%, 126%, and 129% of control at 50%, 80% and 100% VO_2 max, respectively (Table 7, Fig. 16). Phy administration produced 130%, 145% and 156% of control hemoglobin at the corresponding time points related to each exercise level. Phy and concurrent exercise showed 140%, 170%, and 186% of control hemoglobin at 50%, 80% and 100% VO_2 max, respectively (Fig. 16). These results indicate that Phy and concurrent acute exercise decreased the hemoglobin content as compared to exercise alone.

Hematocrit:

Acute exercise produced hematocrit values of 111%, 114% and 115% of control at 50%, 80% and 100% VO_2 max, respectively. Phy administration alone did not produce any significant effect on hematocrit from 10-30 min. Phy followed by concurrent acute exercise produced hematocrit values of 96%, 97% and 104% of control at 50%, 80% and 100% VO_2 max, respectively (Table 7, Fig. 17).

In conclusion, Phy administration and acute exercise showed a significant interaction with lactate and pyruvate metabolism. Phy administration followed by acute exercise decreased the exercise-induced increase in hemoglobin.

Table 7: Effects of physostigmine administration (70 ug/kg, i.m.) and different levels of concurrent acute exercise on biochemical parameters in rats. Values are mean ± SEM.

Groups	n	Blood hemoglobin g/dl	Blood hematocrit %	Plasma lactate mg/dl	Plasma pyruvate mg/dl
Control	6	7.861 ± 0.923	36.751 ± 0.769	50.941 ± 5.142	0.571 ± 0.048
VO_2 max 50%	4	8.865 ± 1.310	40.951 ± 0.690	50.013 ± 2.234	0.377 ± 0.012
VO_2 max 80%	4	9.937 ± 1.105	41.738 ± 0.781	91.819 ± 3.451	0.974 ± 0.094
VO_2 max 100%	4	10.201 ± 0.982	42.175 ± 0.947	111.829 ± 4.276	1.302 ± 0.102
Phy 10 min	4	10.278 ± 0.906	37.775 ± 0.449	60.869 ± 6.447	0.675 ± 0.098
Phy 20 min	4	11.411 ± 1.034	38.251 ± 1.269	52.044 ± 4.214	0.512 ± 0.032
Phy 30 min	4	12.273 ± 1.337	38.151 ± 1.221	53.073 ± 5.994	0.428 ± 0.044
Phy + VO_2 max 50%	4	11.013 ± 0.858	35.051 ± 1.061	70.341 ± 0.794	0.973 ± 0.169
Phy + VO_2 max 80%	4	13.375 ± 0.699	35.575 ± 1.678	73.481 ± 0.549	0.887 ± 0.221
Phy + VO_2 max 100%	4	14.643 ± 1.029	38.051 ± 0.646	91.271 ± 7.099	0.873 ± 0.080

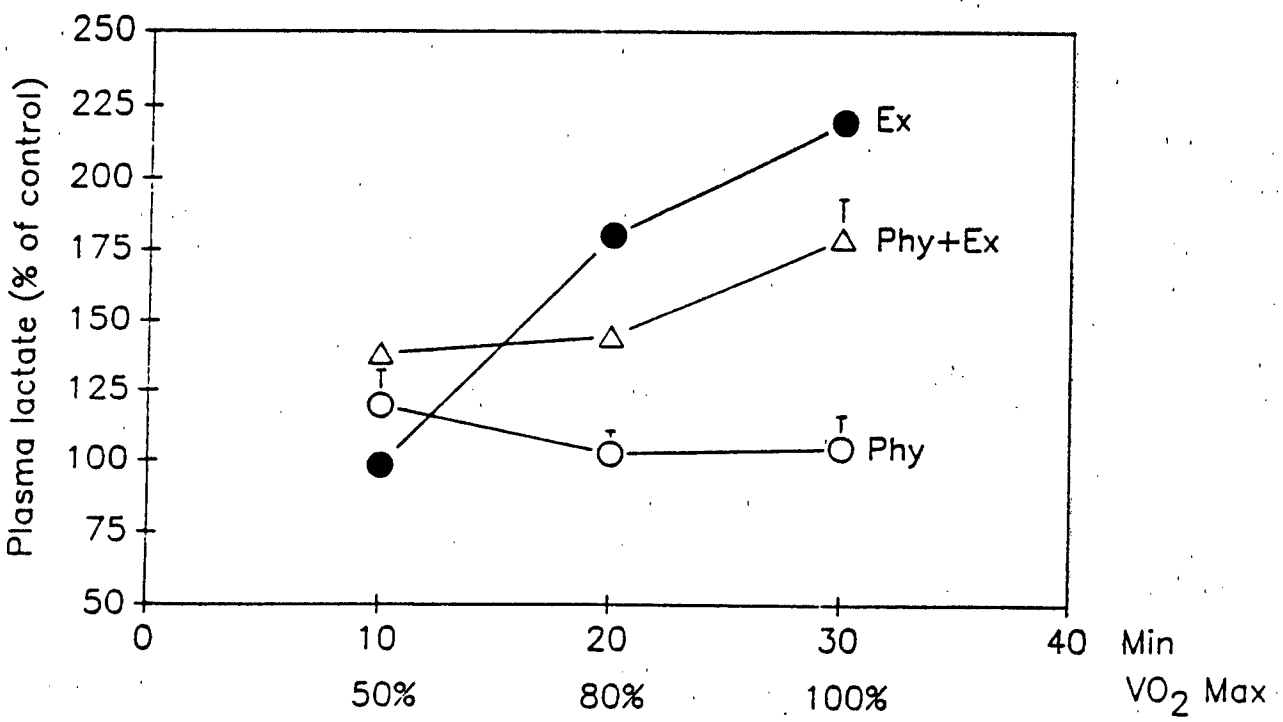

Fig. 14. Effect of physostigmine administration (70 ug/kg, i.m.) and concurrent acute exercise (50%, 80% and 100% VO_2 max) on plasma lactate concentration (% of control) in rats. Values are Mean ± S.E.M.

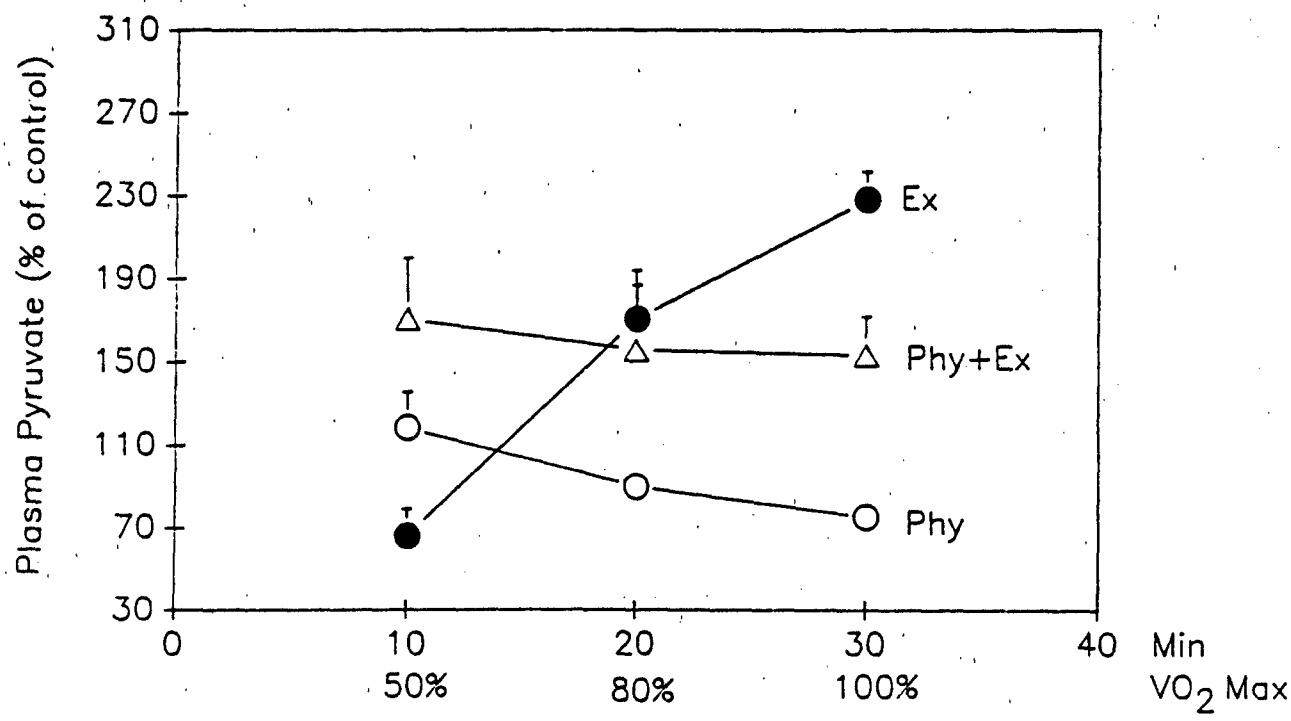

Fig. 15. Effect of physostigmine administration (70 ug/kg, i.m.) and concurrent acute exercise (50%, 80% and 100% VO_2 max) on plasma pyruvate concentration (% of control) in rats. Values are Mean ± S.E.M.

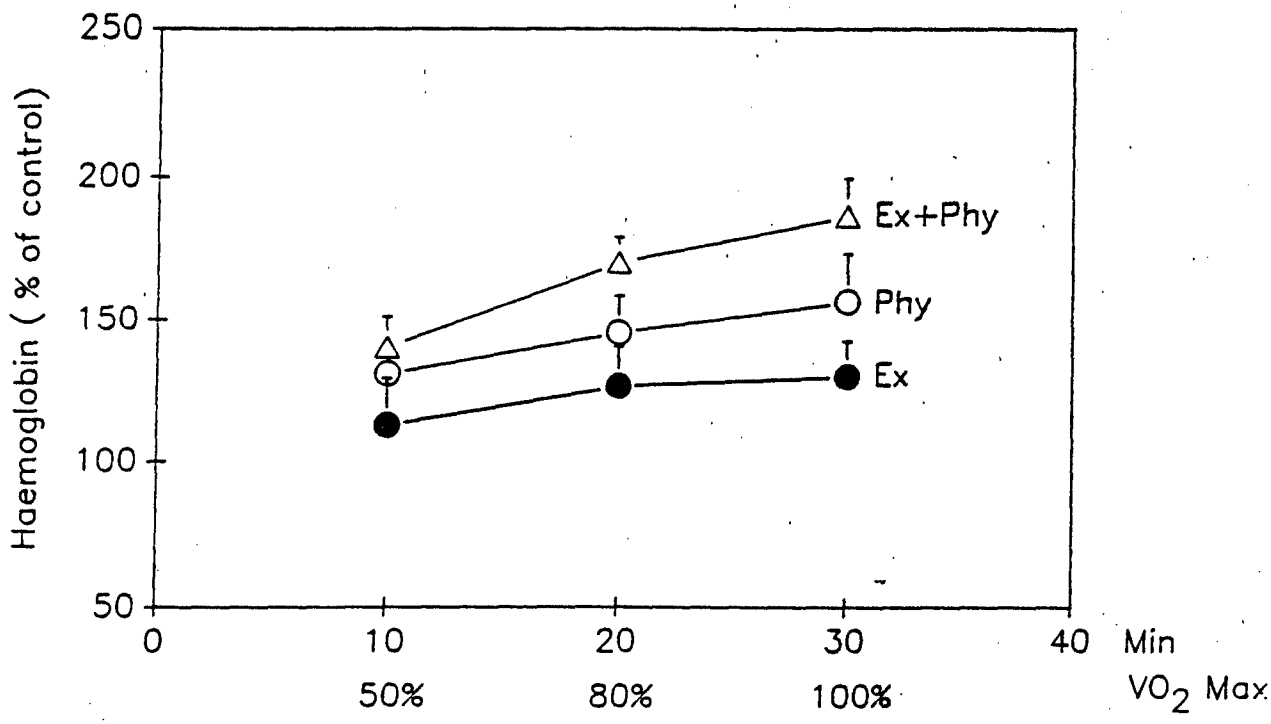

Fig. 16. Effect of physostigmine administration (70 ug/kg, i.m.) and concurrent acute exercise (50%, 80% and 100% VO_2 max) on hemoglobin concentration (% of control) in rats. Values are Mean ± S.E.M.

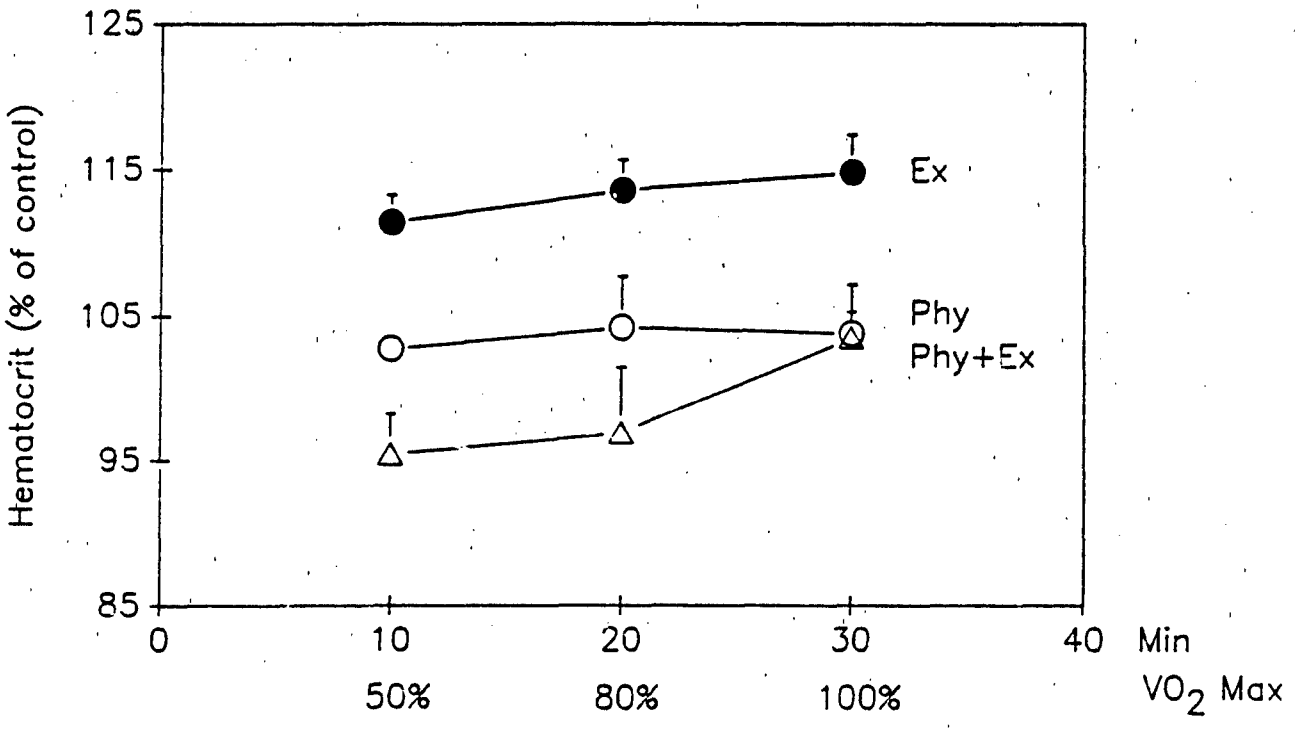

Fig. 17. Effect of physostigmine administration (70 ug/kg, i.m.) and concurrent acute exercise (50%, 80% and 100% VO_2 max) on hematocrit (% of control) in rats. Values are Mean $\pm$ S.E.M.

V. TIME COURSE OF CHOLINESTERASE ACTIVITY IN RBC AND TISSUES AFTER PHYSOSTIGMINE ADMINISTRATION IN TRAINED RATS.

Introduction:

Exercise is one of the important factors which affect the enzymatic sensitivity to a number of compounds acting primarily on the cholinergic system. The degree of enzymatic change depends on the kind and severity of exercise (86,87). However, there is little information with respect to the effects of exercise on ChE activity in RBC and tissues. Physostigmine, a centrally acting anticholinesterase drug, is considered a potential prophylactic agent against organophosphate intoxication. The effect of Phy on work performance is of extreme importance. We have previously shown the dose-dependent inhibition of ChE activity in RBC, brain, heart, diaphragm and thigh muscle by intramuscular administration of physostigmine in doses of 25-500 ug/kg (92). The effect of exercise and Phy administration on ChE activity is not known.

This section describes the effects of endurance training and endurance training followed by Phy administration on the time course of ChE activity in RBC and various tissues of rats.

Materials and Methods:

Rationale:

S.M. Somani, Phil Buckenmeyer, Shirley Baer, Eileen Hurley and research technical experts Jon Holcomb, Dave Adams, Steve Dawson, Mason Colliver and Craig Owens looked into purchasing an exercise treadmill and, in the process, consulted the following firms: Columbus Instrument, Inc., Omnitech, Inc. and Quinton Corp. After careful consideration of performance, efficiency, maintenance, research requirements, cost and capabilities of our technical workshop staff, it was concluded that we should construct the treadmill in our own workshop. The treadmill that we built has nine channels for simultaneous exercise training of rats, and four of these channels can be used in metabolic determination of parameters such os oxygen consumption, carbon dioxide production and heat production. The rats are monitored using an oxyscan analyzer. The primary factor in deciding to construct our treadmill was a cost reduction of approximately 66%, and this treadmill will be used primarily for training the rats.

Channels:

The treadmill unit, which rests on a movable cart (25" x 47.7" x 36"), is composed of nine spearate exercise channels, each measuring 21" x 4.12" x 12.5". Within each channel, oxygen consumption and carbon dioxide production are continuously monitored. All of the channels are identical in design features, each being equipped with its own conveyor belt which can be loosened or tightened by handles on either side of the instrument, and possessing a sampling port at the top of the channel. A tygon tube connects the sampling port to a dryer column containing calcium sulfate (for absorption of excess moisture in expired gases). One of the nine channels differs from the others in that it is composed of a "double ceiling", with a 9 mm space separating the top ceiling from a 5-mm-thick Plexiglas plate which has 64 rounded holes spanning the length of the ceiling of the channel. The non-identical channel serves a basis of a simulated comparison of uniform gas distribution vs. single-entrance port-source delivery. In addition, at the front of each channel there is a black coating which is 8" in coverage for the top and sides of the channel. The remainder of the channels

are clear Plexiglas on the top, sides and back of the channels. This design feature enables the rat to experience a similar habitat under scotopic or low-illumination conditions. The sampling is centrally located in the top of the Plexiglas, ensuring a uniform gas mixture so that more accurate values for oxygen consumption (VO_2), carbon dioxide production (VCO_2), respiratory exchange ratio (RER) and heat production can be monitored by a computerized oxyscan analyzer.

Shocking apparatus:

Within each channel, toward the rear of the treadmill, four plastic circular disc devices are evenly spaced and attached to a metal rod. Each circular disc contains several spiked copper-bushing brushes. The brushes within adjacent discs alternate in polarity, widthwise.

Photocell:

Located immediately in front and to the left of the shocking apparatus in each channel is an infrared photo-relay sensor detector. When a portion of the rat's body crosses the plane of the photocell, the circular discs rotate, allowing the spiked brushes to act as a further stimulus for the rat to maintain the desired speed. The rat's contacting two adjacent brushes of opposite polarity causes a shocking pulse to be administered.

Motor and inclinators:

A one-fourth (1/4) horsepower motor (1750 rpm) drives each channel belt to speeds between 1 and 99.99 meters per minute, while an inclinating device on the side of the treadmill allows for variance of the angle of elevation between 0° and 90°.

Electronic dials and contacts:

The housing for most of the treadmill's electronic functions is a water-tight compartment (so that the conveyor belts can be washed on a regular basis) and resides below the treadmill on a main panel to the right of the instrument. Included within this panel is a main power switch which activates all auxillary switches. In addition, there are two switches related to the shocking apparatus. The first switch turns the mechanism either on or off, while the second sets the intensity of the shock.

Electronic functions:

Within the same panel there are two switches related to controlling the rate of speed. The first switch is an on-off switch which activates all speed functions, while the second switch allows the experimenteer to manually dial in a selected speed. When the speed is manually entered, it is shown in an LED display. An emergency switch is also available for stopping the movement of the entire treadmill if desired.

Capabilities:

The treadmill allows one to simultaneously compare several metabolic parameters in response to different exercise intensities. The inclusion of the "double-ceiling" channel with several minute pores allows comparison of single-pore gas delivery vs. multiple-pore source delivery. The inclinator and speed module can be varied co-jointly to establish a protocol to challenge the rat, while the shocking apparatus and photocell brush setup provide accurate values

for oxygen consumption and carbon dioxide production at submaximal and maximal levels of exercise intensity.

Animals:

Male Sprague-Dawley rats (initial weight 160-200 g) were used. Rats were divided into 4 groups: sedentary control (gr 1), endurance trained (gr 2), physostigmine (gr 3) and endurance trained + physostigmine (gr 4).

Endurance exercise training of rats:

Rats from groups 2 and 4 were trained on a 9-channel motor-driven treadmill (built in our SIU Sch. Med. workshop), utilizing an incremental exercise program 5 days a week for 6 weeks. During this program of exercising, the speed (in m/min), angle of inclination (in degrees), and the duration (in min) of exercise were varied to obtain different levels of exercise intensity.

The exercise endurance training protocol (Table 8) was selected and designed on the basis that a more demanding exercise task would confront each animal as each week of training elapsed. In the first 2 weeks, conveyor belt speeds were 8.2, 15.2, and 19.3 m/min and the angle of inclination was 5°. Exercise duration was 5 min the first week and 10 min the second week at each speed.

In the 3rd and 4th weeks, exercising speeds were 19.3, 26.8 and 30.3 m/min. The duration of exercise was 10 min. The angle of inclination was 5° during the 3rd week and 8° the 4th week.

The final 2 weeks of exercise involved sustaining speeds of 35.4, 40.0 and 43.8 m/min at an 8° angle of inclination for 10 min.

Rats from groups 1 and 3 were not trained, but were maintained under conditions similar to those of the exercised rats. Each rat's weight was recorded daily before the rats were exercised in the treadmill, in order to determine the body weight changes during the entire period of training. Also, metabolic variables (oxygen consumption, respiratory exchange ratio and heat production) were determined every week, in a subgroup of 16 rats from endurance trained rats, to determine whether training has any effect on the maximal oxygen consumption (VO_2 max).

Dosing and sacrificing of rats:

On the day of experiment, trained rats were run on the treadmill for 30 min as per protocol of the 6th week. These exercised rats (gr 2) were decapitated at 5, 15, 30 and 60 min. Rats from gr 4 were also exercised as per protocol of the 6th week, and then administered Phy 70 ug/kg, i.m. and sacrificed at 2, 5, 10, 15, 30, 45 and 60 min. Eight rats from gr 1 received saline and were sacrificed. Rats from gr 3 were administered Phy 70 ug/kg, i.m. and were sacrificed at 2, 5, 10, 15, 30, 45 and 60 min. Four to six rats were sacrificed at each time point in gr 2, 3 and 4. Blood and tissues (brain, heart, diaphragm, thigh muscle, lung, liver, adrenal gland and kidney) were collected for determination of ChE activity.

Table 8. Endurance-training protocol for exercising rats.

Week	Belt Speeds	Angle of Inclination (degrees)	Duration at each speed (min)
1	8.2, 15.2, 19.3	5	5
2	8.2, 15.2, 19.3	5	10
3	19.3, 26.8, 30.3	5	10
4	19.3, 26.8, 30.3	8	10
5	35.4, 40.0, 43.8	8	10
6	35.4, 40.0, 43.8	8	10

Results:

Effect of Training on ChE Activity in RBC:

Endurance training alone produced ChE activity of 66%, 75% and 76% of control at 5, 30 and 60 min, respectively (Fig. 18). Phy administration (70 ug/kg, i.m.) produced 72%, 67%, 73%, 77% and 79% of control ChE activity at 2, 10, 15, 45 and 60 min, respectively. Endurance training followed by Phy administration did not produce much change up to 30 min as compared to changes produced by Phy alone or exercise alone. However, endurance training followed by Phy administration slightly increased the effect of Phy on ChE activity, especially at 60 min (Fig. 18).

Effect of training on ChE activity in brain:

Endurance training alone produced a slight decrease in ChE activity of brain, which varied from 92-96% of control from 5 to 60 min (Fig. 19). Phy produced a time-dependent effect on ChE up to 10 min (55% of control), which started recovering back and reached to 78% of control at 60 min. Endurance training and Phy administration increased the Phy-induced decrease in ChE activity at all time points from 2 to 60 min (Fig. 19). Results indicate that endurance training modifies the effect of Phy on ChE activity, resulting in further decrease in ChE activity.

Effect of training on ChE activity in heart:

Endurance training alone showed a % control ChE activity of 85-75% from 5 to 60 min (Fig. 20). Phy produced peak effect on ChE activity at 15 min (68% of control). Then, 30 min onwards the recovery of inhibited ChE started, and at 60 min it showed 89% of control ChE. Endurance training followed by Phy further decreased the % control ChE activity to 71%, 61% and 73% at 2, 15 and 60 min, respectively (Fig. 20).

Effect of training on ChE activity in diaphragm:

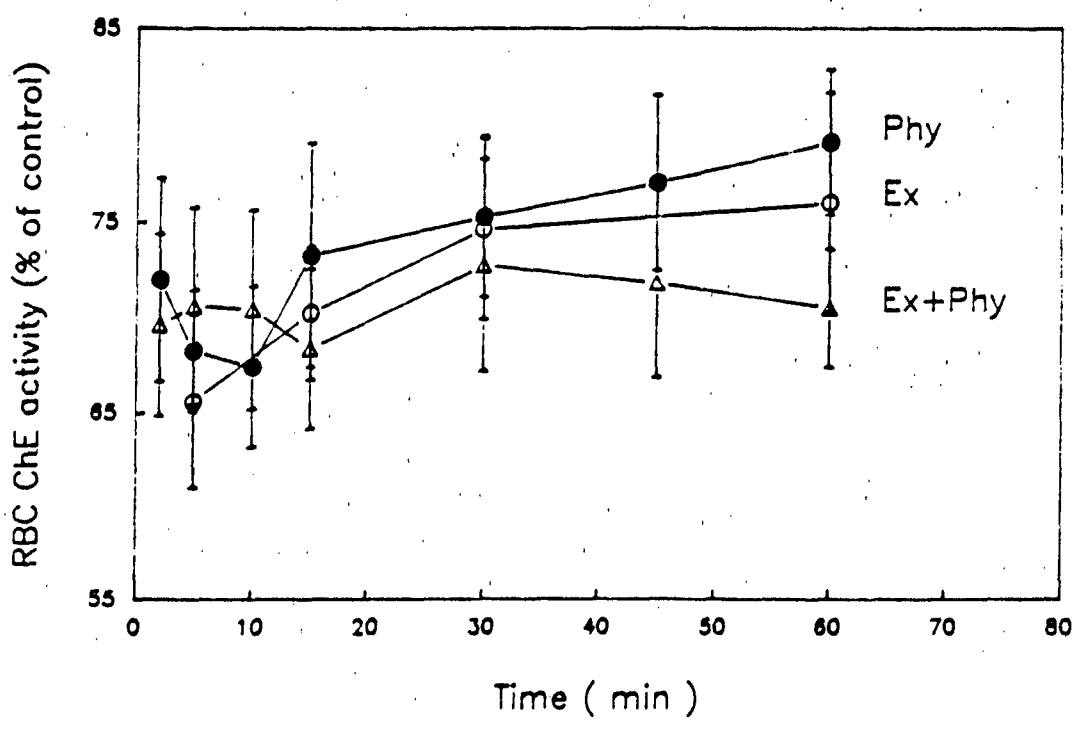

Fig. 18. Time dependent effect of physostigmine (70 ug/kg, i.m.) on % control ChE activity in RBC of trained rats. Values are Mean ± S.E.M.

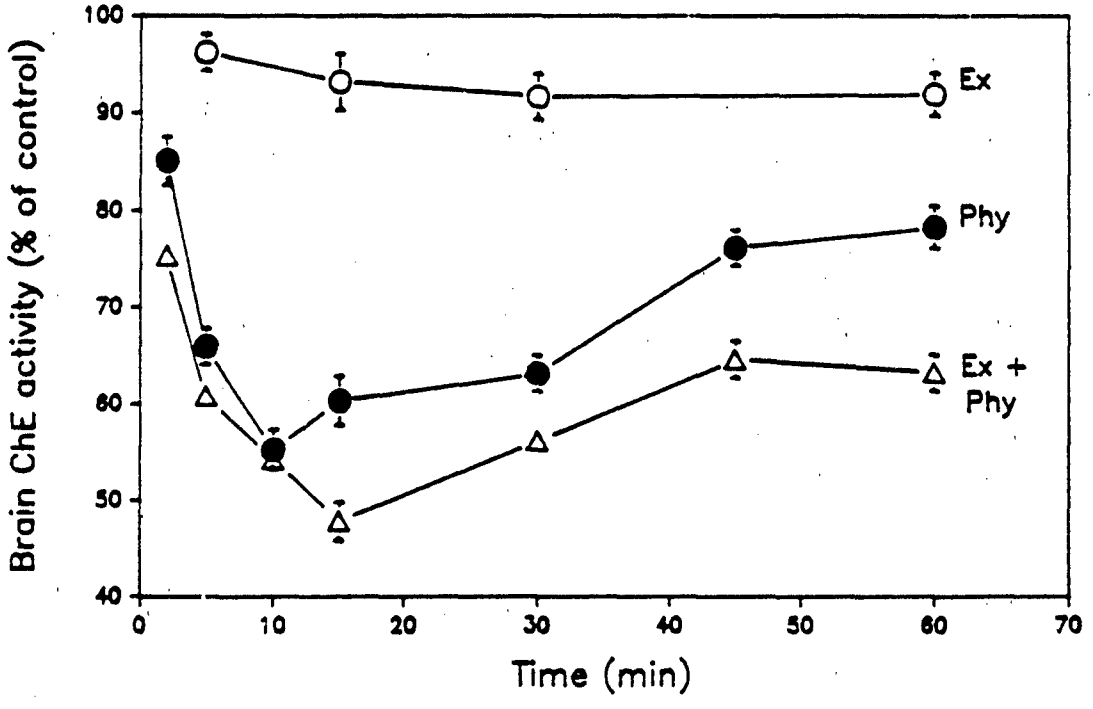

Fig. 19. Time dependent effect of physostigmine (70 ug/kg, i.m.) on % control ChE activity in brain of trained rats. Values are Mean ± S.E.M.

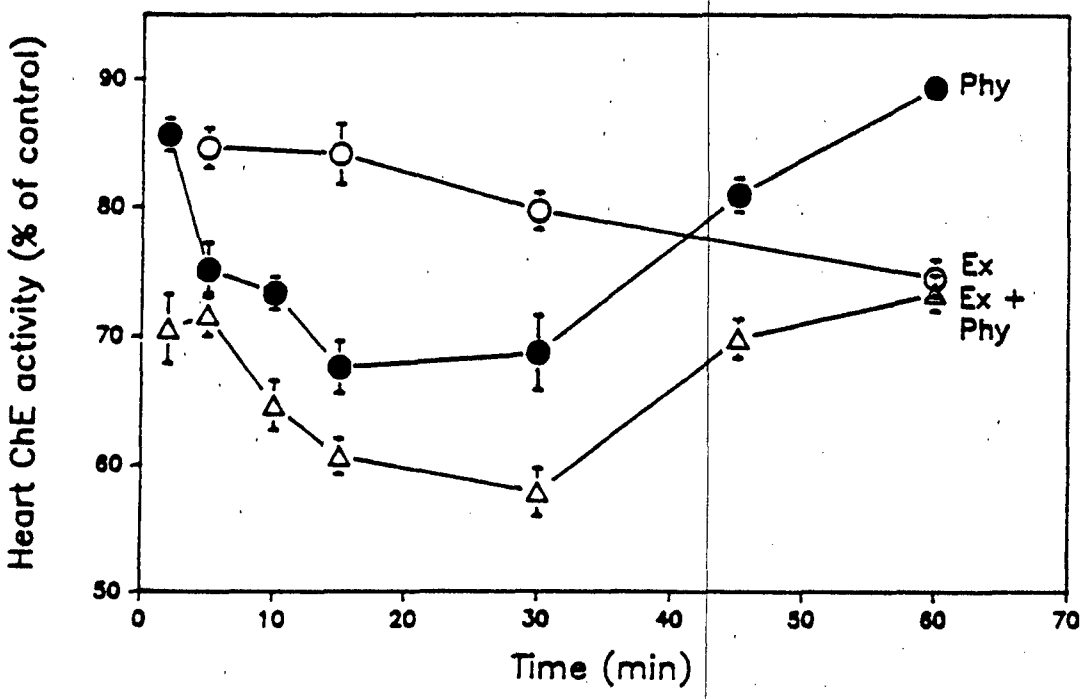

Fig. 20. Time dependent effect of physostigmine (70 ug/kg, i.m.) on % control ChE activity in heart of trained rats. Values are Mean ± S.E.M.

ChE activity in diaphragm of endurance trained rat was 85-78% of control from 5 to 60 min. Phy administration showed ChE activity of 90%, 66% and 81% of control at 2, 15 and 60 min, respectively. Endurance training and Phy further decreased the % control ChE activity to 81-68 from 2 to 60 min (Fig. 21).

Effect of training on ChE activity in muscle:

The endurance training showed a % control ChE activity of 89-76 from 5 to 60 min (Fig. 22). Phy produced % control ChE activity of 86-67 from 2-30 min and then slowly recovered to 83% of control at 60 min. Endurance training followed by Phy further decreased the ChE activity at various time points as compared to ChE activity with Phy alone (Fig. 22).

VO_2, RER and heat production:

These metabolic variables were determined once a week in the trained rats so that the effect of training could be observed. The VO_2 max was found to be decreased with the increase in duration of exercise (Fig. 23). The initial VO_2 max was 77.1 ml/kg/min which came down to 68.7 ml/kg/min on the 43rd day (Fig. 23). The heat production increased with increase in duration of exercise as compared to resting stage. Exercise did not produce any significant change in RER.

In conclusion, exercise alone produced a slight decrease in ChE activity in RBC, brain, heart, diaphragm and thigh muscle at various time points. Physostigmine administration (70 ug/kg, i.m.) decreased ChE activity in RBC and various tissues 30-40% within 10-30 min, which recovered to almost control level (80-90%) within 60 min. Exercise and Phy administration decreased the ChE activity further than Phy alone or exercise alone did. These results indicate that exercise modifies the pharmacodynamic effect of Phy on ChE activity.

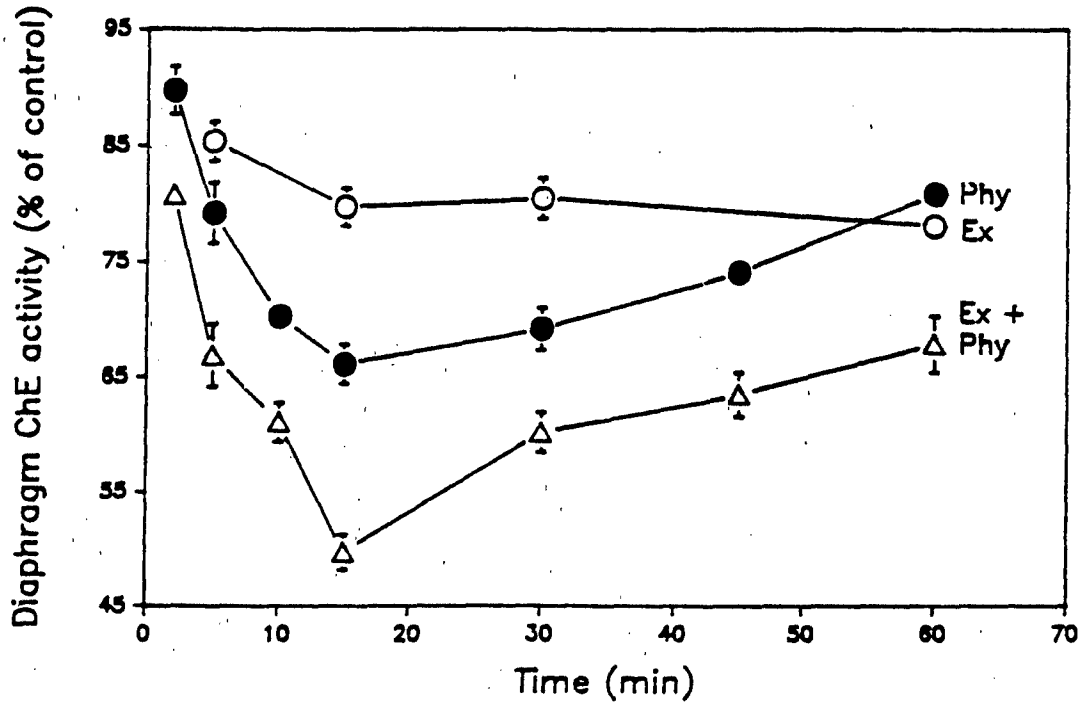

Fig. 21. Time dependent effect of physostigmine (70 ug/kg, i.m.) on % control ChE activity in diaphragm of trained rats. Values are Mean ± S.E.M.

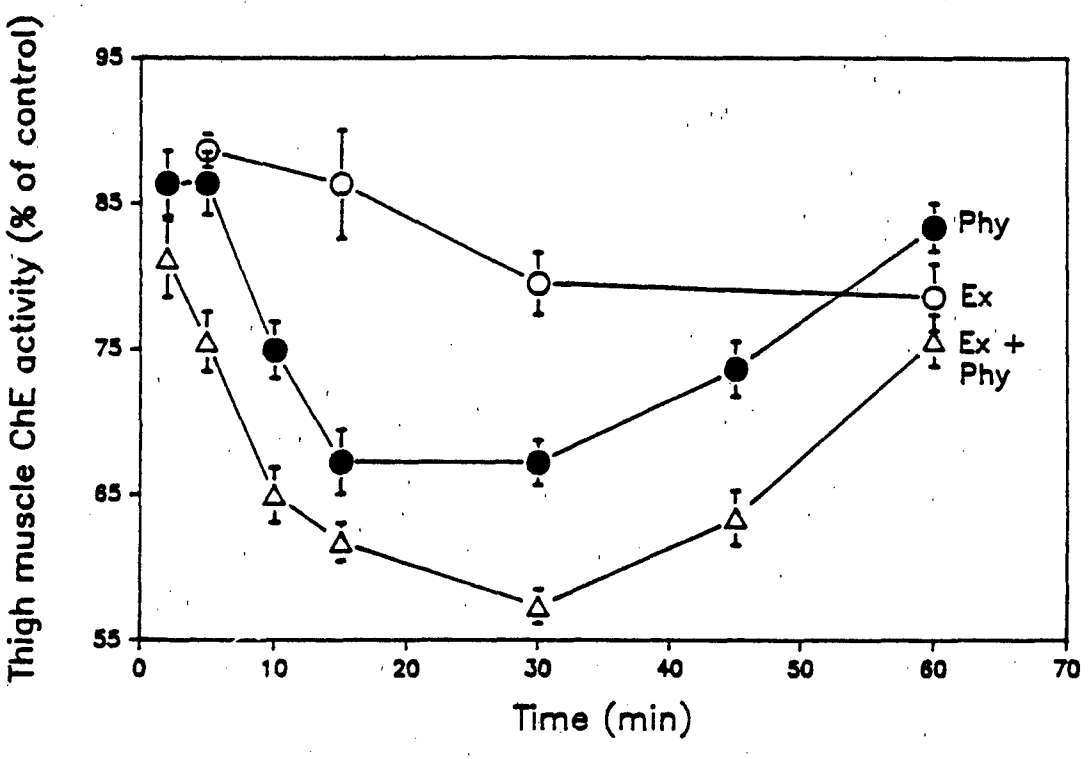

Fig. 22. Time dependent effect of physostigmine (70 ug/kg, i.m.) on % control ChE activity in thigh muscle of trained rats. Values are Mean ± S.E.M.

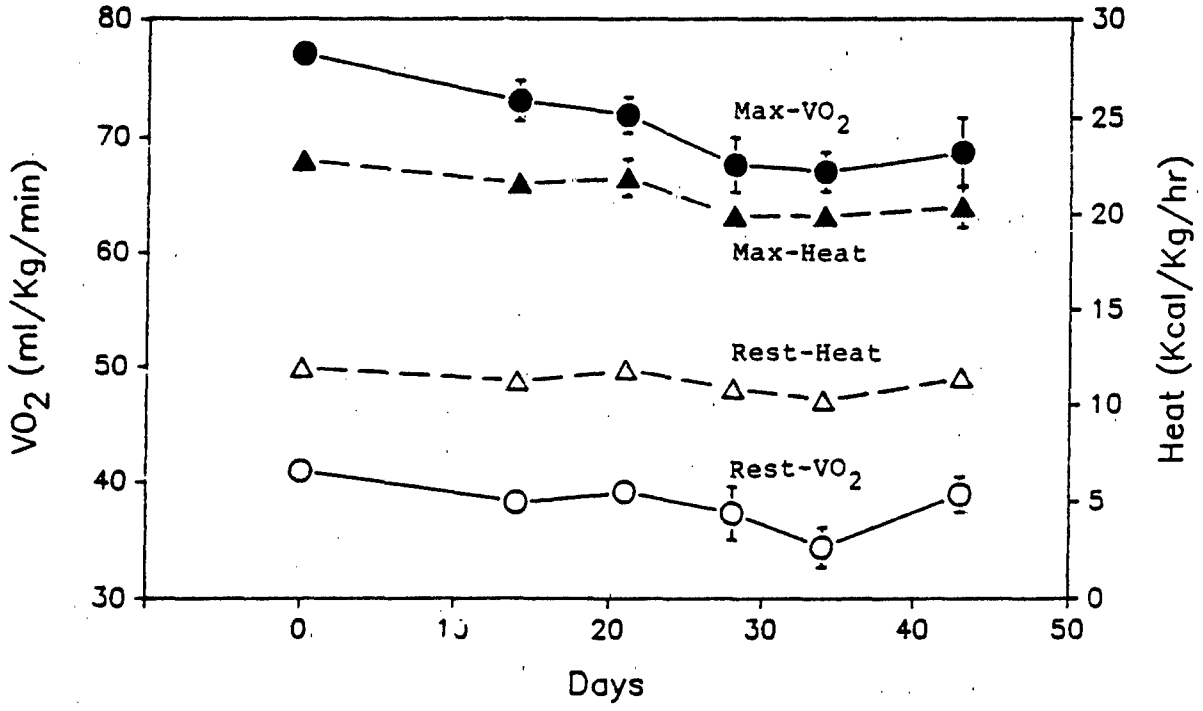

Fig. 23. Effect of training on oxygen consumption (VO_2) and heat production in rats. Values are Mean ± S.E.M.

VI. EFFECT OF PHYSOSTIGMINE ON TIME COURSE OF BLOOD BIOCHEMICAL PARAMETERS IN TRAINED RATS.

Physical activity creates a demand for energy. The energy output from the working muscle may be as much as 120 times higher than the output from the muscle at rest (107). During less intense, but sustained exercise, the energy requirement increases 20 to 30 times above the requirement during rest. The immediate energy is provided by high-energy phosphate ATP and the creatinine phosphate (CP) system. Only 5 mmol of ATP and 15 mmol of CP are stored per kilogram of tissue; this store provides energy only for brief periods of exercise.

The levels of blood lactate provide a fairly objective indication of the relative strenuousness of exercise and may also reflect the adequacy of the recovery process (107). Some authors used blood lactate as an index of anaerobic glycolysis (108). In moderate ventilated phased exercise, lactic acid is recycled to other energy-rich compounds. Prolonged work can be performed in a phased manner allowing recovery time by periodic monitoring of lactate and pyruvate levels in blood and tissues. Lactate and pyruvate assays are widely used as measures of oxygen debt (109).

The effects of training on hemoglobin concentration are not clear. An increase in hemoglobin in trained exercise group was reported by Astrand and Rodal (110). Decrease in hemoglobin in trained subjects was also reported by Oscai et al. (111). Some authors reported no change in hemoglobin during training (112). An increase in hemoglobin concentration would be an advantage for trained subjects because the increased hemoglobin would deliver more oxygen to the tissues as required during exercise.

Very little information is available on interaction of drugs such as Phy and exercise on lactate and pyruvate levels. Pyruvate and lactate are the major sources for the production of the acetyl group, which combines with choline to produce acetylcholine (112). Thus, this interaction may affect the formation of acetylcholine. This section of the report describes the effect of training, physostigmine and training + physostigmine on lactate, pyruvate and hemoglobin.

Materials and Methods:

Chemicals:

This has been described in Section V.

Animals:

Male Sprague-Dawley rats (initial weight 160-200 g) were used. Rats were divided into 4 groups: trained exercise (gr. 1), physostigmine dose (gr. 2), trained exercise + physostigmine (gr. 3) and sedentary control (gr. 4).

Exercise training of rats:

This has been described in Section V.

Dosing and sacrificing of rats:

This has been described in Section V.

Plasma lactate, pyruvate, blood hemoglobin and hematocrit:

Methods for determination of plasma lactate, pyruvate, blood hemoglobin and hematocrit have been described in Section IV.

Results:

Lactate:

In the trained exercise group, lactate concentration was 128% of control at 5 min and decreased to control level in 60 min (Table 9, Fig. 24). In the physostigmine-administered group, significantly high concentrations (221% and 167% of control) of lactate were observed at 2- and 5-min time periods. After 10 min plasma lactate concentrations were steady at control level, and little variation was observed. In the trained exercise and physostigmine-administered rats, the lactate concentration was 138% of control at 2 min and decreased to 95% of control at 10 min (Table 9, Fig. 24). There was no significant change up to 30 min.

Pyruvate:

In the trained exercise group, 159% of control pyruvate was observed at 5 min (Table 10, Fig. 25), but pyruvate concentration showed a decreasing trend. In the physostigmine-injected group, a significantly high concentration (.259 $\pm$ .065 mmol/L; 214% of control) of pyruvate was observed at the 2-min time point, which decreased to 140% of control at 10 min and further decreased to 72% of control at 60 min. In trained exercised and physostigmine-injected rats, pyruvate concentration was 145% of control at 2 min and decreased to 74% of control at 60 min.

Hemoglobin:

In the trained exercised group, hemoglobin increased to to 113% of control at 30 min and then decreased slowly (Table 11, Fig. 26). In the physostigmine-administered group, high hemoglobin concentration (129% of control) was observed at the initial time period. It decreased to control level at 30 min. In the exercised and physostigmine-administered group, hemoglobin was 112% of control at 2 min. Hemoglobin decreased to 89% of control at 5 min and returned to normal level after 15 min.

Hematocrit:

Hematocrits have shown no significant changes in any of the groups studied. A steady state was observed in the exercised group (Table 12, Fig. 27). In the physostigmine-administered group, hematocrit decreased to 85% of control at 10 min and returned to control levels at 45 min. In the trained exercise and physostigmine-administered group, hematocrit was 96% of control at 2 min and decreased to 90% of control at 30 min. Then, a steady state was observed up to 60 min.

Discussion:

In the trained exercise group, the plasma lactate showed a peak concentration of 6.502 mmol/L (128% of control) at 5 min and decreased to 5.64 mmol/L at 15 min. Many authors reported that the blood lactate increased during the first few minutes of recovery and that lactate reached peak concentration after the end of exercise (108, 114). The rats were endurance trained for several weeks before sacrifice. The training increases the blood lactate threshold and also

Table 9. Effects of training, physostigmine, and training + physostigmine on time course of plasma lactate levels in rats.

Lactate concentration mmol/L

Time (Min)	Training	Physostigmine	Training + Physostigmine
2		11.215 ± 0.757	7.040 ± 0.860
5	6.502 ± 0.754	8.482 ± 1.677	5.887 ± .004
10		5.116 ± .386	4.813 ± 0.272
15	5.646 ± 0.985	5.316 ± 0.675	5.008 ± 0.410
30	5.483 ± 0.613	4.883 ± 0.664	6.714 ± 1.450
45		5.288 ± 0.282	4.903 ± 0.352
60	5.124 ± 0.613	4.863 ± 0.609	4.362 ± 0.291

Control: 5.05 ± 0.307 mmol of lactate/L ± SE, N = 4

Table 10. Effects of training, physostigmine, and training + physostigmine on time course of plasma pyruvate levels in rats.

Pyruvate concentration mmol/L

Time (Min)	Training	Physostigmine	Training + Physostigmine
2		0.259±0.065	0.176±0.041
5	0.192±0.045	0.349±0.095	0.176±0.062
10		0.170±0.029	0.160 ±0.012
15	0.147±0.013	0.163±0.040	0.080 ±0.007
30	0.134±0.024	0.124±0.064	0.097±0.015
45	0.117±0.022	0.104±0.016	
60	0.118±0.012	0.087±0.033	0.090±0.022

Control: 0.1212 ± 0.0221 mmol of pyruvate/L ± SE, N = 4

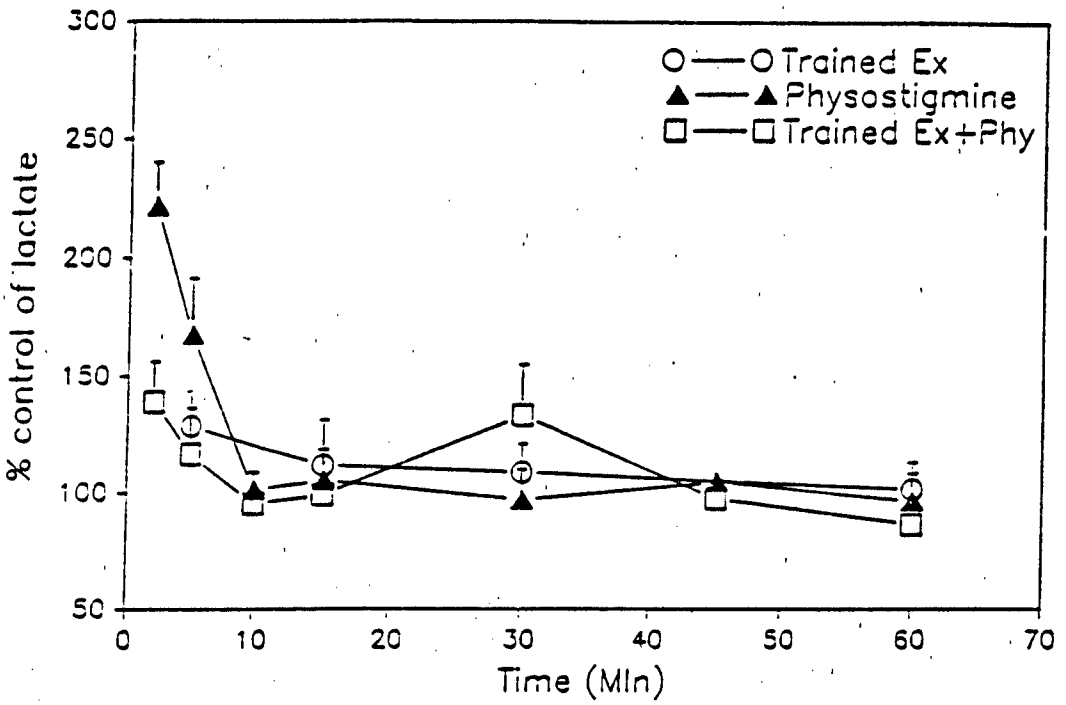

Fig. 24. Percent control of plasma lactate concentrations (Mean ± S.E.M) as a function of time after chronic exercise. Control = 5.055 ± 0.307 mmol/L. N = 4.

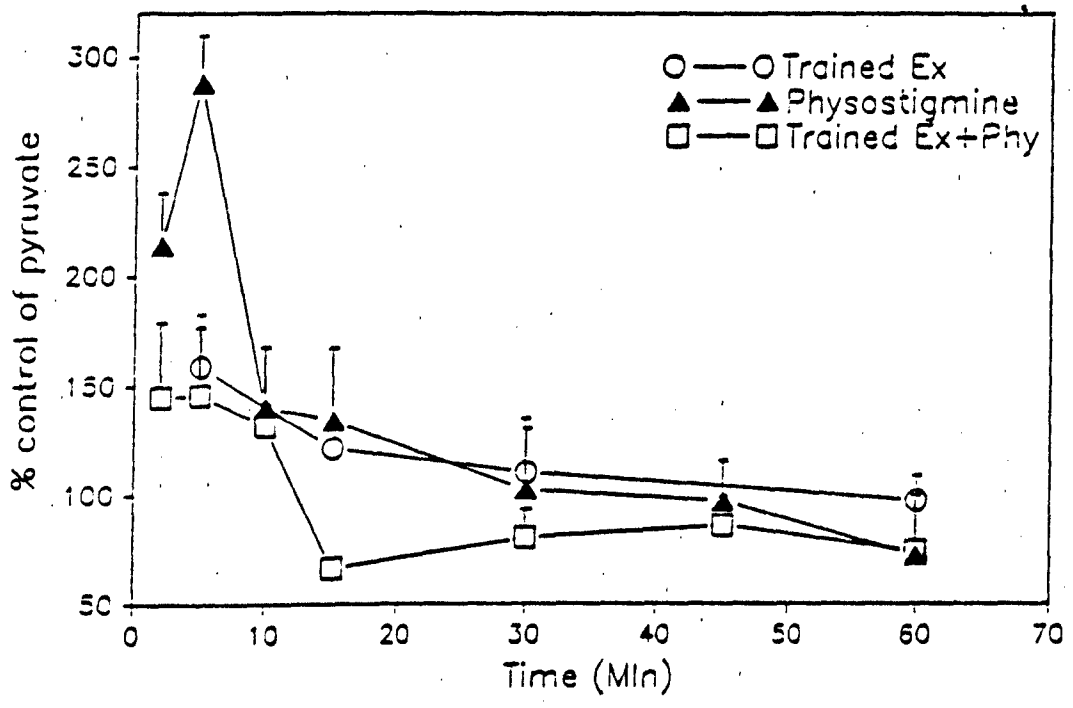

Fig. 25. Percent controls of plasma pyruvate concentration (Mean ± S.E.M.) as a function of time after chronic exercise. Control = 0.121 ± 0.222 mmol/L. N = 4.

Table 11. Effects of training, physostigmine, and training + physostigmine on time course of blood hemoglobin levels in rats.

Hemoglobin concentration in g/dL

Time (Min)	Training	Physostigmine	Training + Physostigmine
2		15.971±0.386	14.005±0.895
5	10.235±0.744	16.503±0.831	11.003±0.588
10		13.430±0.839	13.860±0.812
15	12.032±0.865	16.279±0.619	11.264±0.869
30	13.836±1.197	12.517±0.562	13.060±1.794
45		12.242±0.758	13.041±0.837
60	11.553±0.917	12.264±1.848	14.096±0.822

Control=12.314±1.219 g/dL of blood ± SE, N = 4.

Table 12. Effects of training, physostigmine, and training + physostigmine on time course of blood hematocrit levels in rats.

Hematocrit in percent

Time Min	Training	Physostigmine	Training + Physostigmine
2		43.58±2.63	42.53±2.24
5	43.17±2.50	42.70±1.40	43.22±1.30
10		39.00±2.65	43.06±2.07
15	43.93±1.63	42.60±1.40	42.05±1.03
30	43.76±1.13	39.21±2.89	40.00±1.50
45		42.16±1.27	43.42±0.74
60	43.47±1.74	42.20±0.65	41.46±0.71

Control=43.98±2.08 percent ± SE, N = 4.

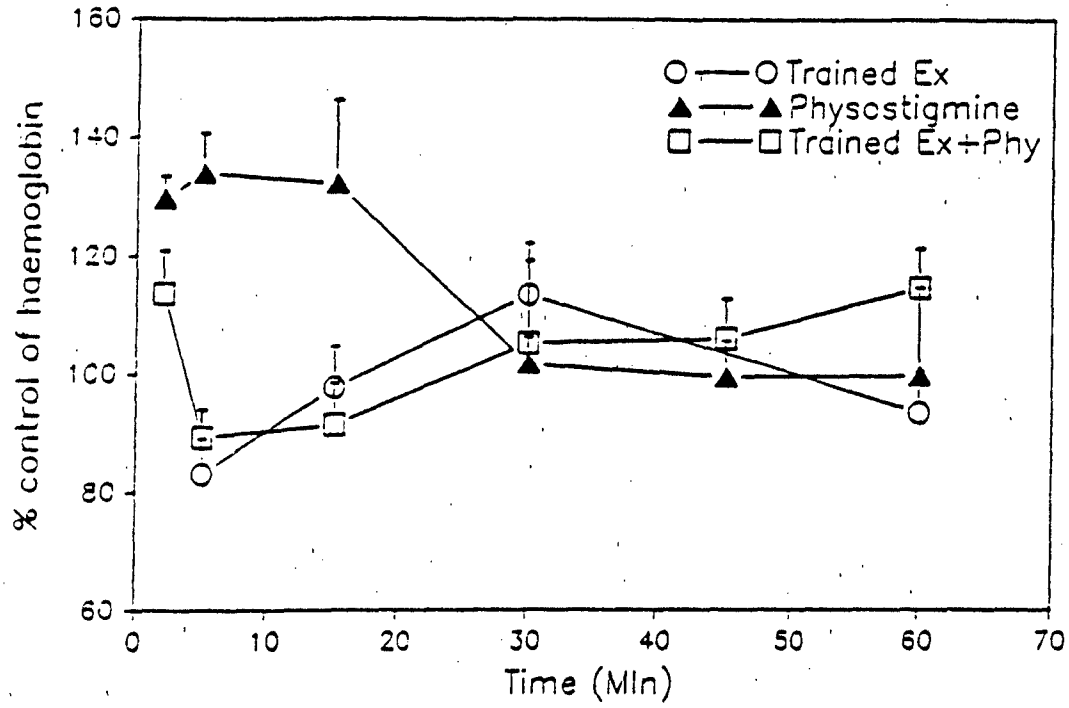

Fig. 26. Percent controls of blood hemoglobin levels (Mean ± S.E.M.) as a function of time after chronic exercise. Control = 12.314 ± 1.219 g/dL. N = 4.

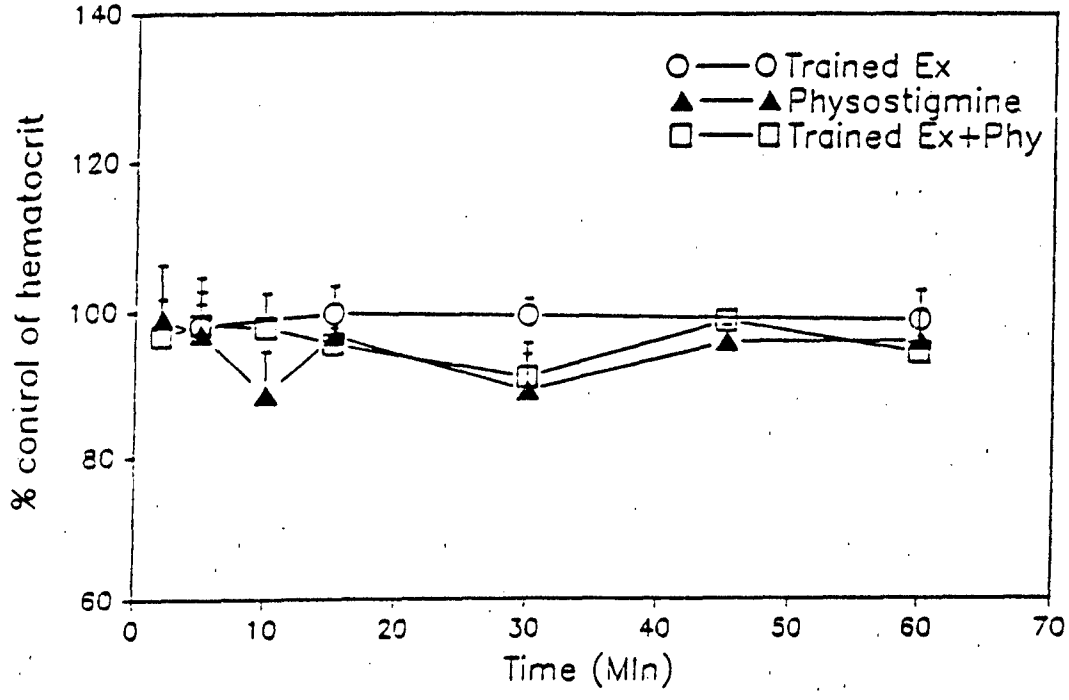

Fig. 27. Percent controls of blood hematocrit levels (Mean ± S.E.M.) as a function of time after chronic exercise. Control = 43.981 ± 2.086 percent. N = 4.

favors decreased production of lactic acid (115) as well as a more rapid rate of its removal (116). Cellular adaptations with training would also enhance the rate of lactate clearance (117). The lower lactate level observed in the trained rats may be due to less production of lactate and to an accelerated rate of lactate removal. Accelerated rate of lactate removal was reported in trained animals (117). The data for lactate production in untrained rats can be found in Section IV, Table 7. In the physostigmine-administered group, significantly high concentrations (11.2 mmol/L) were observed at the initial time points. Compared to untrained counterparts, trained animals showed both greater lactate oxidation and greater conversion of the lactate to glucose (118). Physostigmine was administered to untrained rats. Physostigmine may create a kind of stress condition. This may have triggered the lactate production. Since the animals were not trained, lactate was produced at a greater-than-normal rate. High concentrations observed at the initial time periods may have been due to the above effect. Physostigmine has a half-life of 15-17 min, and most of the Phy will be removed or metabolized after 15 min. Therefore, the rats were restored to their normal states after 30 min. In the trained exercise + physostigmine-administered group the trend was similar to that of the trained group. The rats were trained for 6 weeks, and they may have developed an adaptation for less lactic acid production (115) and its immediate removal (116). This may be the cause for the constant level of lactate in this group.

At the 2-min time point, 0.192 mmol/L of pyruvate concentration was observed in plasma of trained rats. The rate of pyruvate utilization during aerobic exercise is controlled both by the rate of pyruvate dehydrogenase activity and by the rate of flux through the citric acid cycle (119). Pyruvate dehydrogenase is regulated by both allosteric modulation of the reaction products, acetyl CoA and NADH, and by a phosphorylation-dephosphorylation of the enzyme by kinase and phosphatase, which are tightly associated with the enzyme complex (120). It was reported that the rates of formation and metabolism of pyruvate increase during exercise (119). Our results confirm the above situation. The rats were sacrificed after exercise at different time points, and the immediate time points show a small increase in pyruvate, which was brought back to normal levels slowly. But in the physostigmine-administered group, significantly high concentrations were observed at the earlier time points. In the trained exercised + physostigmine-administered group, the trend was almost identical to the pyruvate concentration pattern in the exercised group.

Blood hemoglobin showed small changes in its concentration in the groups studied. Small changes in the hemoglobin content during exercise may be due to change in plasma volume. Change in plasma volume during exercise has been reported (121, 122). A small decrease in blood hemoglobin in trained exercised rats may have been due to a plasma shift. In the physostigmine-administered group, hemoglobin concentrations were higher at the initial time points. This may be due to the shift of fluid from blood to interstial places, resulting in higher hemoglobin concentrations in this group. In the trained + physostigmine-administered group hemoglobin concentrations were almost identical to concentrations in the trained exercised group. No significant changes were observed in blood hematocrits in any of the groups studied.

It has been reported that lactate production and utilization is altered by factors such as exercise (123, 124), chemicals (125) and several disorders (126). Blood pyruvate levels are also influenced by a number of disorders such as liver disease, congestive heart failure, muscular dystrophy, thiamin deficiency and neoplastic conditions (127). Blood flow is altered during exercise (128). In the present experiment the rats were sacrificed at different

time points after exercise and drug administration. Normal steady states were reached after 30 min, indicating the time taken for returning to normal state after trained exercise, acute exercise or after drug administration. The results indicate that trained exercise will help the body to cope with stress conditions such as drug adminstration and will help to restore normal conditions sooner than in untrained animals.

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LIST OF ABBREVIATIONS

Acetylcholine	ACh
Acetylcholinesterase	AChE
Butyrylcholinesterase	BuChE
Carbon dioxide production	VCO_2
Centrigrade	°C
Cerebellum	CR
Choline	Ch
Cholinesterase	ChE
Clearance	Cl
Concentration corresponding to half-maximal inhibition	IC_{50}
Correlation-coefficient	r
Cortex	Cx
Creatine phosphate	CP
Curie	Ci
Diisopropylfluorophosphonate	DFP
Disintegration per minute	DPM
Dose corresponding to half-maximal inhibition	ID_{50}
Elimination rate constant	Ke
Elimination rate constant from brain	Kb
Renal elimination rate constant	Ku
Eseroline	Es
Extent of inhibition	I
Extraction ratio	ER
Gram	g
Half-life	$t_{1/2}$
Hepatic clearnce	Cl_H
Hippocampus	Hc
High performance liquid chromotograph, chromatograph, chromotogram	HPLC
Hour	hr
Inhibition	Inh
Intramuscular	i.m.
Intrinsic clearance	Cl_{int}
Intravenous	i.v.
Kilogram	kg
1 kcal/kg x hr	MET
Maximum inhibition	I_{max}
Maximal oxygen consumption	VO_2max
Microliter	ul
Microgram	ug
Milliliter	ml
Millimole	nmole
Minute	min
$Metabolite_1$	M_1
$Metabolite_2$	M_2
$Metabolite_3$	M_3
Milligram	mg

Medulla oblongata	MO
Molar	M
Nanogram	ng
Oxygen consumption	VO_2
Physostigmine	Phy
Radioactivity	RA
Respiratory exchange ratio	RER
Revolutions per minute	rpm
Retention time	Rt
Standard error of mean	S.E.M.
Striatum	Str
Septum	Sep
Trichloroacetic acid	TCA
Tritiated	^3H
Tritiated water	^3H_2O
Ultraviolet	u.v.
Versus	vs.
Volume of distribution	Vd
Volume	v
Weight	w